



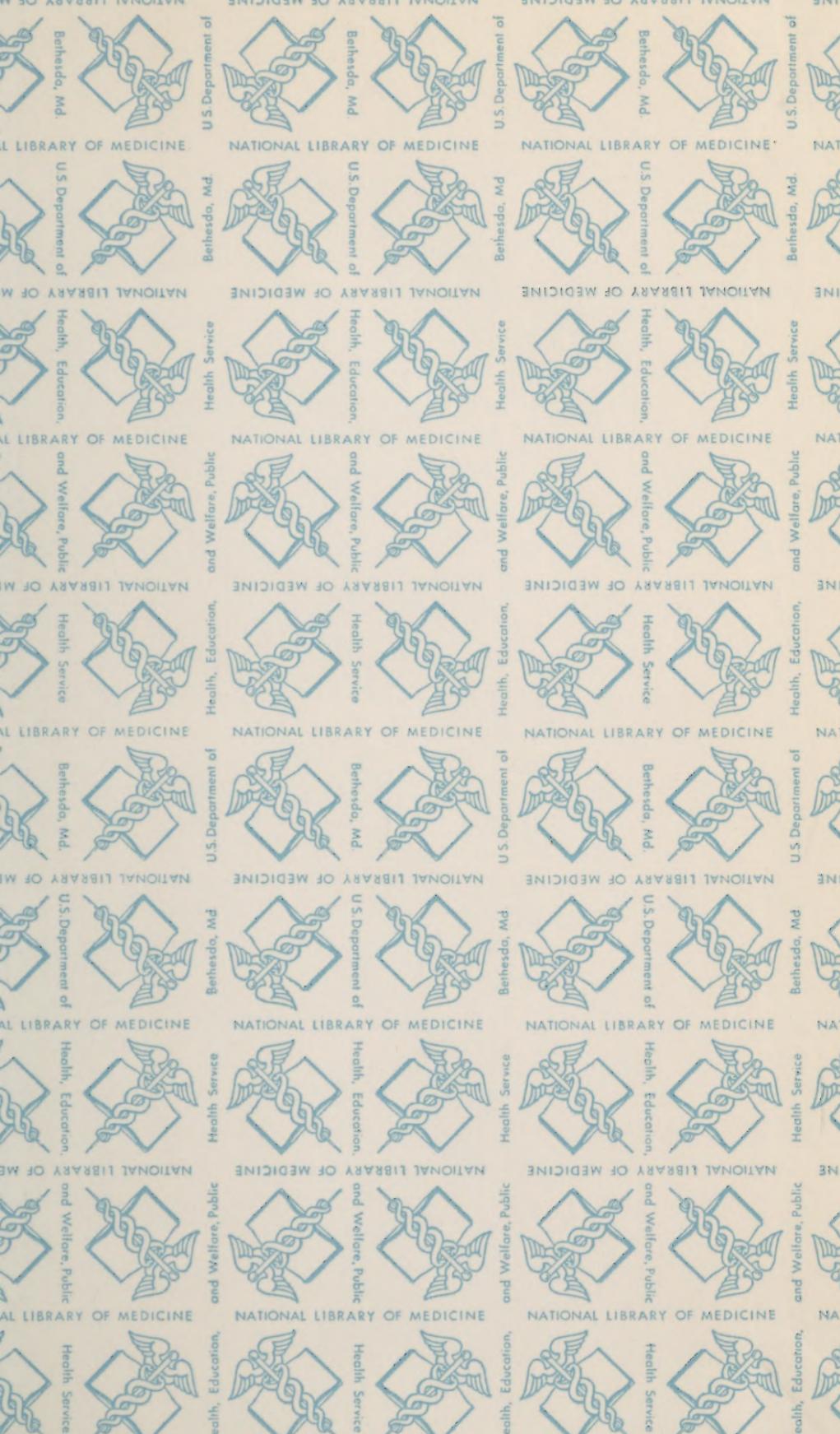
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# THE CHEMISTRY OF TUBERCULOSIS

BEING A COMPILATION AND CRITICAL REVIEW  
OF EXISTING KNOWLEDGE ON THE CHEMISTRY OF THE  
TUBERCLE BACILLUS AND ITS PRODUCTS

THE CHEMICAL CHANGES AND PROCESSES IN THE HOST

THE CHEMICAL ASPECTS OF THE TREATMENT  
OF TUBERCULOSIS

BY

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BY ESMOND R. LONG

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## PREFACE

In developing the systematic work on the chemotherapy of tuberculosis that has been carried on in the Otho S. A. Sprague Memorial Institute since its organization in 1911, it has been necessary to accumulate and review critically preceding and contemporaneous work on the chemical aspects of tuberculosis and related problems. This is widely scattered in medical, chemical and bacteriological literature, so that the task has been by no means simple. In order that the results of our own labors may be made available, and to spare repetition of the same labor by others, this monograph is published, in the hope that it may facilitate in some degree the study of the problems of tuberculosis and serve as a reasonably complete reference work on the chemical processes of interest to the student of this disease.

There exists already a review of the early literature of this topic, in Ott's "Die Chemische Pathologie der Tuberculose," published in 1903. Since that time biological chemistry has made great progress, and unfortunately much of the chemical work recorded in the nineteenth century presents so many errors of method that it has little or no present value. We have drawn freely on Ott's book for the earlier work in the field, and on account of the existence of this work have not considered it necessary to go into the historical aspects of the subject. Some topics of interest in connection with the study of tuberculosis are covered in Wells' Chemical Pathology, from which we have also extracted material, notably on Amyloidosis, Addison's Disease and Calcification.

If this book fulfils its purpose as an aid to the study of tuberculosis it is our intention to keep the subject matter up to date in new editions. For this purpose we shall be grateful for information of errors or omissions of importance in this first edition.

THE AUTHORS.



## **SECTION I**

### **THE CHEMISTRY OF ACID-FAST BACTERIA**

BY

**ESMOND R. LONG**



## CHAPTER I

### THE CHEMICAL COMPOSITION OF THE TUBERCLE BACILLUS

The chemical composition of the tubercle bacillus has been more extensively investigated than that of any other micro-organism. The immense amount of chemical research which has accumulated was initiated by two striking discoveries, made within six years after Koch's isolation of the bacillus, from which the great majority of subsequent investigations have taken departure, viz., Ehrlich's discovery of its acid-fastness and Hammerschlag's finding of its high content of fatty material. To a very large extent subsequent research has been concerned with an elaboration of the second of these and an explanation of the first, and in many cases with an attempt to correlate the two.<sup>1</sup>

#### LIPINS<sup>2</sup>

Hammerschlag<sup>3</sup> in 1888 analyzed two crops of tubercle bacilli, each one of which was a mixture of cultures from two sources, broth and agar, with the addition of hay infusion cultures in one case. Calculation from his published figures indicates that the organism averaged 86 per cent water, and that 28.2 per cent of the dry weight in one case and 26.2 per cent in the other, was material soluble in alcohol and ether. Of the dry fat-free residue 8 per cent remained as ash on ignition. The elementary composition of the fat-free substance, calculated on an ash-free basis, was as follows: C, 51.62 per cent; H, 8.07 per cent; N, 9.09 per cent.

In the further investigation of the alcohol-ether extract this material was saponified with barium hydrate and the mixture

<sup>1</sup> The earlier literature is discussed by E. R. Baldwin in the English translation of Cornet's "Tuberculosis," 1904.

<sup>2</sup> In this book the term "lipins" is used in the comprehensive sense advocated by Gies and Rosenbloom, to include the entire group of fats and lipoids.

<sup>3</sup> Sitzungsber. d. k. Akad. d. Wissensch., Dec. 13, 1888.

neutralized with a current of carbon dioxide and filtered. The filtrate from the barium carbonate and fatty acids was evaporated to dryness and the residue ashed. Phosphorus was detected in the residue, its finding indicating to Hammerschlag the presence of lecithin.

The mixture of barium carbonate, fatty acids and barium soaps was acidified with hydrochloric acid and extracted with ether. The lipin material obtained from this extract melted at 63° and was believed by its investigator, on the basis of this relatively high melting point, to be a mixture of stearic and palmitic acids, containing very little oleic acid. That it unquestionably contained other substances, not fatty acid in nature, but of the group of waxes, will be brought out below.

Hammerschlag's investigation was not confined to the fatty material, but its most conspicuous finding was the high lipin content of the organism, which thus early in tuberculosis research served as a point of distinction between the infective agent of this disease and other pathogenic organisms known at the time, which were known not to possess so high a fat content. It has been but a short step further to associate this peculiarity with other distinctive features, as acid-fastness and the marked resistance of the organism to intra-corporeal lysis.

Although Hammerschlag is usually credited with the discovery of the organism's high lipin content, a relationship between acid fastness and a high fat content of the medium had been recorded two years earlier by Bienstock<sup>4</sup> and Gottstein,<sup>5</sup> whose work will be considered in the chapter on acid-fastness to follow.

Investigations on the chemical composition of the tubercle bacillus made prior to 1900 have been reviewed in detail by Jolles in Ott's Chemical Pathology of Tuberculosis and only a few contributions before that date need be referred to here. De Schweinitz and Dorset,<sup>6</sup> taking departure from Cramer's statement that the composition of a given bacterium was practically constant while it was grown on a rich medium but varied considerably when the conditions of assimilation were difficult,

<sup>4</sup> Fortschritte d. Medizin, 1886 (4), 193.

<sup>5</sup> Ibid., 1886 (4), 252.

<sup>6</sup> Jour. Amer. Chem. Soc., 1895 (17), 605.

examined the tubercle bacilli grown on glycerol bouillon and on a synthetic medium containing asparagin, ammonium phosphate and glycerol. In their limited number of observations they apparently did not verify Cramer's finding. Their figures on lipins for these organisms and for *B. mallei* grown on broth for comparison are as follows:

|                    | BROTH<br>TUBERCLE BACILLI | ASPARAGIN<br>TUBERCLE BACILLI | BROTH <i>B. MALLEI</i> |
|--------------------|---------------------------|-------------------------------|------------------------|
| Ether extract..... | 39.29-40.80               | 37.57-38.34                   | 7.78-8.17              |

Their figures for lipins are, as seen, considerably higher than Hammerschlag's. The variation in the amount of fatty material in tubercle bacilli grown on the two media was small. Broth-grown glanders bacilli contained only one-fifth as much lipin as tubercle bacilli. A qualitative examination of the "fat" was not made at that time, but shortly subsequently<sup>7</sup> they saponified 3.5 grams of the ether extract with sodium hydroxide, obtaining a hard soap difficultly soluble in water. Distillation with acid yielded a small amount of volatile acid with a pungent odor, which was not identified. Fractional crystallization of the fatty acids gave principally an acid melting at 62°, apparently palmitic acid. Small yields of two other acids, which were thought to be lauric and arachidic acids, were obtained.

A little later, Edwin Klebs,<sup>8</sup> in his research on fractions of the tubercle bacillus which might be useful immunizing substances, obtained a yield of 22 per cent of the dry weight as fatty substance, and made the statement that this fatty material was, like the tubercle bacillus itself, acid-fast, and probably responsible for the peculiar tinctorial properties of the tubercle bacillus.

Aronson<sup>9</sup> apparently was the first to emphasize the fact that a large part of the ether and alcohol extractable substance of the tubercle bacillus was not true fat but a waxy material. His results also indicated that 17 per cent of the ether-alcohol extract was in the form of free fatty acids, the total amount of neutral

<sup>7</sup> Cent. f. Bakt., 1896 (19), 707.

<sup>8</sup> Cent. f. Bakt., 1896 (20), 488.

<sup>9</sup> Berl. klin. Woch., 1898 (35) 484.

fat thus being a comparatively small part of the 20 to 25 per cent of the tubercle bacillus which he found extractable with fat solvents. He asserted that the non-saponifiable residue from this wax was an alcohol, soluble in ether, ligroin and acetone, giving an acetate with acetic anhydride, and not giving the Liebermann reaction for cholesterol. He noted the fact, to which attention had already been called by Klebs, that the tubercle bacillus "fat" was acid-fast. He states that other organisms also contain fat, but not in anything like the same amount. For instance, 5 per cent was obtained from the bodies of diphtheria bacilli, which, in contradistinction to that obtained from the tubercle bacillus, was non-acid-fast.

Because of his finding that long extraction with alcohol and ether left the bacilli intact and acid-fast, Aronson was led to conclude that much of the fatty material thus removed was between the bodies of the bacilli and not in them. That the wax, however, really was responsible for their acid-fastness he did not doubt, inasmuch as repeated extraction with alcohol and ether containing 1 per cent hydrochloric acid rendered them completely non-acid-fast. He believed that a difficultly extractable lipoid shell was thus responsible for the failure of the bacillus to stain readily, and for its resistance, once stained, to acid decolorization.

A later investigation by Aronson<sup>10</sup> brought out the fact that the wax, which was highly resistant to hydrolysis, could be saponified by heating with strong alcoholic potash under pressure in a sealed tube. The non-saponifiable residue was strongly acid-fast. Its alcohol nature was demonstrated by acetic anhydride solution. It did not give the reactions of cholesterol.

One of the most careful studies ever made on the lipins of the tubercle bacillus was that by Kresling.<sup>11</sup> This author was the first to determine the physical constants. He used tubercle bacilli grown on glycerol-peptone broth, killed by heat, washed to the disappearance of the glycerol, dried at 40°, and extracted with ether, alcohol and chloroform. The lipin extract was about 40 per cent of the dry weight, with a melting point for the mixed

<sup>10</sup> Berl. klin. Woch., 1910 (47), 1617.

<sup>11</sup> Cent. f. Bakt., 1901 (30), 897.

"fat" of 46°. The following figures give the results of his gross partition of the material thus secured. They are slightly modified from those published by Kresling himself, as the latter's figures are for bacilli still containing 3.94 per cent water. The percentage figures for anhydrous bacilli are: Ash, 2.65; nitrogen, 8.92; protein,<sup>12</sup> 55.74; fatty substances, 40.57.

The most important constants for the lipin extract were as follows: Acid number, 23.08; Reichert-Meissel number, 2.00; Saponification number, 60.70; Esterification number, 37.62; Iodin number, 9.82. The finding of an acid number of 23.08, that is, the number of milligrams of potassium hydroxide required to neutralize 1 gram of fat, confirmed Aronson's report of a relatively high content of free fatty acid. By taking up the neutralized product in water, filtering, and reacidifying, Kresling obtained fatty acids corresponding to 14.38 per cent of the total lipin. The melting point of the mixed acids was 53.5°.

The Reichert-Meissel number of 2.00 indicated that very little of the material was in the form of volatile acid. Butyric acid was, however, recognized in the distillate by its odor. The saponification number, 60.70, the number of milligrams of potassium hydroxide required to neutralize the free fatty acid as well as that derived from neutral fats or other esters, is surprisingly low, so low in fact as to indicate almost certainly that saponification was not complete. As Goris points out (see below) fats ordinarily have a saponification number of 175 to 250, while waxes have a much lower one, but not below 79 (Carnauba wax). The tubercle bacillus lipin, being a mixture of the two, should give a figure in between. The esterification number, or number of milligrams of potassium hydroxide used up in saponifying neutral fats and esters, the difference, that is, between the saponification number and the acid number, is accordingly the figure which is to be considered at fault. The iodin number of 9.72, the amount of iodin in milligrams absorbed by 100 mgm. of fat, is low as compared with most fats (goose fat 70, lard 50 to 70, mutton tallow 25 to 46) and about on the order of that of beeswax, which is commonly given as 8 to 11. It indicates the presence of only a moderate number of unsaturated carbon atoms.

<sup>12</sup> Calculated by multiplying the figure for nitrogen by 6.25.

After the free fatty acids were removed, Kresling obtained 77.25 per cent of the total fatty extract as neutral fat and other esters of fatty acids. From this material on prolonged hydrolysis in benzene with sodium ethylate, a yield of 39.10 per cent of the total lipins was obtained as non-saponifiable alcohol, which melted at 44°. This alcohol did not give the reactions for cholesterol. On the basis of the amount of phosphate in the lipoid material of this analysis Kresling calculated a content of 0.16 per cent lecithin for tubercle bacillus fat.

Bulloch and Macleod,<sup>13</sup> whose article may profitably be consulted for a concise review of the literature on the lipins of the tubercle bacillus up to 1904, with the information on the non-saponifiable portion of tubercle bacillus fat accumulated by Aronson and Kresling at hand, attempted to correlate the wax content with acid-fastness. Their work, which will be considered in more detail in the chapter on acid-fastness, emphasized among other findings the very great difficulty of extracting all of the fatty material from tubercle bacilli with the ordinary solvents, and secondly the great resistance of the wax obtained from it to saponification.

Their material, which consisted of several kilograms of killed tubercle bacilli, a by-product of tuberculin manufacture, was extracted with methylated spirit containing 1 per cent hydrochloric acid (compare Aronson's mixture). This extract deposited on cooling a white precipitate which melted at 47° and was found on appropriate testing to be strongly acid-fast. It will be referred to in that connection later. On saponification of this substance with sodium ethylate in benzene a white powder was obtained which was found by J. Lewkowitsch to have the following constants: Melting point, 44.50; iodin number, 9.39; saponification number, 49.40; increase in weight on acetylation, 1.2 per cent; saponification value of acetylated product, 69.0.

The increase in weight resulting from treatment with acetic anhydride of course indicates that a certain amount of uncombined alcohol was present, the OH group being free to react with the acetyl radical, but a saponification number of 49.40 made it certain that a considerable share of the material was still in the

<sup>13</sup> Jour. Hyg., 1904 (4), 1.

ester state. The pure alcohol of this material was obtained, however, by repeated treatment with boiling alcoholic potash, and final extraction with petroleum ether.

The methylated spirit extract was also examined for its content of fatty acids. An ether extract of it was blackened by osmic acid and had an iodine number of 38.7 to 40.8, which indicated a much higher degree of unsaturation for this fraction than Kresling found for the total lipin. The acrolein reaction for glycerol was positive; therefore a certain proportion of the material was in the form of neutral fat. Phosphorus, and accordingly lecithin was absent, and although the Salkowski reaction was positive, no crystals of cholesterol could be obtained. On saponifying the fat of the ether extract, acidifying, and crystallizing the fatty acids from hot 70 per cent alcohol, an oil was obtained which instantly blackened osmic acid and was thought to be oleic acid. By using the Salkowski technique for fractional crystallization of fatty acids in an alcoholic solution of lead acetate, the authors obtained acids melting between 54° and 57° which were thought to be myristinic and isocetinic acids, although the evidence seems rather doubtful, the amounts obtained being too small for analysis. No mention is made of palmitic acid, which was considered by the earlier investigators, especially De Schweinitz and Dorset, to make up the bulk of the fatty acid present.

With the completion of Kresling's and Bulloch and Macleod's work on the lipin content of the tubercle bacillus, the point had been made and proved that a considerable portion of the body of the bacillus is in the form of an alcohol, or alcohols, of high molecular weight, in combination with a limited series of fatty acids, and that the signal behavior of this organism to decolorizing agents is in some way related to the presence of this alcohol.

Numerous details, however, have been added since these investigations, which, in turn pave the way for further research. Dorset and Emery<sup>14</sup> confirmed previous assertions on the alcohol nature of the non-saponifiable portion of the wax. Baudran<sup>15</sup> precipitated from the ether extract with acetone a substance which he considered a distearic lecithin. He claimed to have

<sup>14</sup> Cent. f. Bakt. Ref. 1906 (37), 363.

<sup>15</sup> Compt. Rend. Acad. Sci. 1906 (142), 657.

recognized olein, palmitin and stearin among the true fats of the extract. Fontes<sup>16</sup> employed xylene as the extracting solvent and precipitated from it with absolute alcohol a yellowish white waxy powder, melting at 53.5°, probably practically identical with the wax isolated by various other methods by the investigators cited above. Auclair and Paris<sup>17</sup> fractionated the total lipins in alcohol, ether, and chloroform, noting that coloring material, lecithin and fatty acids dissolved in the first of these, while the other two took out substances of waxy nature which they believed to be allied to cholesterol. Panzer<sup>18</sup> attempted to solve the question which had repeatedly been raised concerning the presence of cholesterol in the waxy material. The majority of reports, based on the Salkowski and Liebermann reactions, indicated that cholesterol was absent. Panzer, using the digitonin method of Windaus (on the ether-alcohol extract of only 2.7 grams of dry bacilli) confirmed this view. He did obtain a lipoid which was precipitated by digitonin, but this substance, small in amount, when separated from the digitonin, failed to give any of the cholesterol color reactions.

Kozniewski<sup>19</sup> reopened the question of the nature of the waxy material as indicated by its saponification number. Using acetone as extracting solvent, he obtained 21.38 per cent of the dry weight from tubercle bacilli of the human type, and 8 to 10 per cent more from bovine type microorganisms. The saponification number of the waxy residue left on evaporation of the acetone, was 123.2, much higher than Kresling's figures, and probably approximating much more closely the correct number for this difficultly saponifiable material. Kozniewski's figure on iodin absorption was 9.92 per cent, practically identical with the figures obtained by Kresling and by Lewkowitsch on Bulloch and Macleod's material.

A valuable contribution to the chemistry of the bacillus, including a consideration of other constituents than lipins, was that

<sup>16</sup> Cent. f. Bakt., 1909 (49), 317.

<sup>17</sup> Arch. de méd. exp., 1907 (19), 129.

<sup>18</sup> Zeit. physiol. Chem., 1912 (78), 414.

<sup>19</sup> Bull. internat. acad. sci. Cracovie, Series A, 1912, 942. (Through Chem. Abstr., 1913 (7), 1527.)

of Tamura,<sup>20</sup> who also correlated the wax content with acid-fastness, examining a saprophytic acid-fast organism, *Mycobacterium lacticola*, for comparison with the tubercle bacillus. Tamura obtained from ether extracts of these two microorganisms, on precipitation with acetone, a substance containing phosphorus and nitrogen, presumably of the general class of "lecithins," or more properly "phospholipins." That this material, however, was not true lecithin, which is a monoamino-monophosphatid, was indicated by the P:N ratio, which is 1:1 in lecithin. Tamura's figures for analysis were:

|        | TUBERCLE BACILLUS | MYCOBACTERIUM LACTICOLA |
|--------|-------------------|-------------------------|
| P..... | 2.98 per cent     | 2.80 per cent           |
| N..... | 2.69 per cent     | 2.60 per cent           |
| P:N*   | 1:1.99            | 1:2.06                  |

\* On basis of atomic weights.

This result indicated to Tamura that he was dealing with a diamino-monophosphatid. He cites Thudichum as having found such compounds in the brain and elsewhere. He refers to Kresling's finding of a small amount of lecithin in the tubercle bacillus, but points out that Kresling's sole basis for the assertion was the finding of  $P_2O_5$  in the lipin material, a result which merely indicated that some phosphatid was present.

The problem of the nature of the wax present in the tubercle bacillus was attacked by Tamura by a somewhat different method than that used by previous investigators, the essential feature of which was acid hydrolysis. Recognizing the difficult extractability of the wax in the first place, he triturated the bacilli in a mortar in 66.6 per cent sulphuric acid (by volume) as a preliminary measure. The triturate was then diluted with water and the fine suspension of protein and lipoids collected and dried. It was then treated fourteen hours with boiling 25 per cent sulphuric acid, to the disappearance of the biuret reaction. A waxy mass separated on top. This wax melted at 46° but on repeated crystallizations from hot alcohol a snow white product

<sup>20</sup> Zeit. physiol. Chem., 1913 (87), 85.

with a constant melting point of 66° was obtained. To this substance Tamura gives the name "mykol" considering it a pure alcohol. The same product was obtained from both micro-organisms. As will be brought out later it was associated in the mind of its discoverer with acid-fastness. This product, which undoubtedly is the same as that described by Aronson, Kresling, Bulloch and Macleod, Dorset and Emery, Auclair and Paris, Kozniewski and others, but probably isolated in a state closer to purity by Tamura, was found to give the following empiric analysis:

|                                      | C<br><i>per cent</i> | H<br><i>per cent</i> |
|--------------------------------------|----------------------|----------------------|
| Tubercle bacillus.....               | 82.46                | 13.30                |
| <i>Mycobacterium lacticola</i> ..... | 82.56                | 13.53                |

The balance presumably was oxygen; and as nitrogen, phosphorus and sulphur, as well as inorganic bases, were absent, the formula  $C_{29}H_{56}O$  was assigned to the product. Confirmation of this empirical formula and at the same time of its alcohol nature was secured by brominating the compound prepared from *Mycobacterium lacticola*, the product containing 16 per cent of bromine, which corresponds exactly with the calculated amount of bromine in the compound  $C_{29}H_{56}O \cdot Br$ . This brom-alcohol on repeated crystallization from hot alcohol melted at 56°. An iodo-alcohol melting at 58° was also prepared.

In the attempt to acetylate the compound a substance with an acetyl number of 75.67 was obtained. As  $C_{29}H_{55}O \cdot OC \cdot CH_3$  calls for an acetyl number of 121, acetylation was considered incomplete. More success was obtained in benzoylating it. A benzoyl ester melting at 57° was prepared, which analyzed C 82.30 per cent, H 11.48 per cent. Calculation for  $C_{29}H_{55} \cdot O \cdot OC \cdot C_6H_5$  gives C 82.36 per cent, H 11.53 per cent. Mykol may thus be considered an alcohol, of the general group of sterols, of which cholesterol is the best known member. Tamura's preparation of it differed from cholesterol, however, not only in its melting point of 66° (cholesterol = 150°) but also in that it did not give the play of colors given by cholesterol in the sulphuric acid and acetic anhydride reactions.

Bürger<sup>21</sup> studied the lipins of human type tubercle bacilli grown on protein free media and dried *in vacuo*; 240 grams of bacilli were extracted with various solvents, acetone furnishing the largest yield, 36 per cent. Ether removed among other things an aromatic substance with the characteristic odor of tubercle bacilli, which gave the ferric chloride and other reactions for salicylic aldehyde. Petroleum ether removed a mixture of substances of which a partial separation could be made with hot alcohol. An alcohol-insoluble compound, melting at 37°, decolorized bromine and analyzed for C<sub>19</sub>H<sub>36</sub>O. An alcohol-soluble substance melting at 50° also decolorized bromine and analyzed for C<sub>15</sub>H<sub>28</sub>O. He also obtained a substance soluble in hot alcohol, insoluble in cold, which occasionally crystallized like cholesterol and was doubly refractive under the polariscope, but which was negative to the acetic anhydride and sulphuric acid tests for cholesterol. It was nitrogen and phosphorus free, analyzing for carbon 82.96 per cent and hydrogen 13.63 per cent. The figures resemble those for the mykol of Tamura, but the difference in melting points was vast, mykol melting at 66°, while Bürger's product fused at 230°. On chilling a hot alcohol extract of the bacilli a whole series of substances was precipitated, with decreasing melting points. A substance was obtained precipitating from alcohol at 16° and melting, but not sharply, between 175° and 198°, and another insoluble in alcohol at 4°, melting at 140°. Bürger thinks these constituents are probably very variable, their amount depending on such factors as the medium of growth and age of the culture.

From an assembling of known facts concerning tubercle bacillus lipin, Bürger concludes that it resembles plant waxes and fats, which according to Czapek, consist of esters of fatty acids and alcohols of high molecular weight. In contrast to glycerides these compounds are broken down with difficulty and must play little part in metabolism. They may be thought of as protective to tissues, like the cerolipoids in which xerophytic plants are rich. The tubercle bacillus apparently contains waxy esters formed by fatty acids of the character C<sub>n</sub>H<sub>2n</sub>O<sub>2</sub>, from lauric to palmitic acids, and alcohols of the character C<sub>n</sub>H<sub>2n-2</sub>O, such as C<sub>15</sub>H<sub>28</sub>O, C<sub>19</sub>H<sub>36</sub>O and C<sub>29</sub>H<sub>56</sub>O.

<sup>21</sup> Biochem. Zeit., 1916 (78), 155.

Agulhon and Frouin<sup>22</sup> believe that the phosphorus-containing compound is a complex mixture of the class of jecorins, or glyco-phospho-lipins. They found that on hydrolysis it broke up into glycerol, fatty acids, a base analogous to choline, and a gum which on further hydrolysis yielded glucose.

One of the most elaborate pieces of work ever done on the lipins of the tubercle bacillus is embodied in the report of Goris.<sup>23</sup> His paper may be consulted for a review of the literature, especially on the subject of the lipins, and for many details concerning the fractions encountered in lipid analysis of the bacillus. He used large quantities of a mixture of human and bovine type bacilli grown on glycerol broth, killed at 110°, filtered, washed, thoroughly dried and triturated. Approximately 40 per cent of the dried material dissolved in chloroform. The crude lipoid melted at 42°. Its saponification number was 124.4, acid number 37.0, and esterification number 87.4. The Hehner number (sum of insoluble fatty acids and non-saponifiable lipoids) was 69.77 and the iodine number 16.22. Four partitions were made of the chloroform solution. First, a substance which proved to be insoluble in ether separated out on concentration of the chloroform. This substance Goris calls *hyalinol*, referring to its glassy physical appearance. It made up 1 per cent of the dry weight of the bacilli, 2.5 per cent of the lipoid. It melted at 175°, softening between 160° and 165°. It was hard and elastic, and quite insoluble in water, alcohol, acetone and ether. On purification by repeated solution in chloroform and precipitation with ether, it analyzed as follows: C, 55.5 per cent; H, 7.15 per cent; O by difference, 37.35 per cent. On saponification it yielded an unknown substance and a mixture of crotonic and isocrotonic acids.

The mother liquor left on removal of the hyalinol was evaporated to dryness and the residue taken up in boiling acetone. A waxy residue was left and on chilling the acetone more deposited. Finally the cold acetone solution was evaporated to dryness. The waxy material insoluble in acetone contained nitrogen and phosphorus and stearic and palmitic acids, apparently in sub-

<sup>22</sup> Bull. Soc. Chim. Biol., 1919 (1), 176.

<sup>23</sup> Ann. Inst. Pasteur, 1920 (34), 497.

stances of the phosphatid group, and lauric acid in combination with an alcohol of high molecular weight, m.p. 64° corresponding to descriptions of "mykol." A sulphur-containing substance and an alcohol melting at 100° were also present.

In the acetone-soluble fraction glycerides of butyric, caproic, oleic, palmitic, stearic, and arachidic acids were identified. Cholesterol could not be identified surely in the lipoids of the tubercle bacillus, although a substance was encountered which in chloroform solution gave a weak coloration like that of cholesterol with sulphuric acid on prolonged standing.

Frouin<sup>24</sup> has investigated the relationship between the composition of the medium and the fat content of bacilli grown upon it, for all three types of tubercle bacilli. He cultivated the organism on the following medium: Potassium acid phosphate, magnesium sulphate and sodium citrate, each 0.1 per cent, asparagine 0.5 per cent, glycerol 5 per cent, and a sugar (glucose, levulose, lactose or maltose). The sugars aided growth to a certain extent and could even replace glycerol when their concentration was increased to 15 per cent. His analyses for fat in several micro-organisms on different media follow, the figures representing the per cent of total lipins in the dry organisms:

| ORIGIN                   | ASPARA-               | ASPARA-                                 | ASPARA-                                 |
|--------------------------|-----------------------|---|---|
|                          | GINE PLUS<br>GLYCEROL | GINE PLUS<br>GLYCEROL<br>AND<br>GLUCOSE | GINE PLUS<br>MANNITOL<br>AND<br>GLUCOSE |
|                          | per cent              | per cent                                | per cent                                |
| Bovine type bacillus     |                       |   |   |
| Vallée.....              | 22.95                 | 23.10                                   | 12.99                                   |
| Burnet.....              | 45.51                 | 43.85                                   | 12.99                                   |
| horse .....              | 42.86                 | 45.51                                   | 8.55                                    |
| Human type bacillus..... | 19.59                 | 21.32                                   | 6.75                                    |
| Avian type bacillus..... | 40.10                 | 39.23                                   | 14.22                                   |

Bovine type bacilli were thus found to be relatively rich in fatty material. Although the addition of glucose to the glycerolated medium of growth greatly increased the yield of bacilli it did not modify the lipin content. Substitution of mannitol and glucose for glycerol on the other hand greatly reduced it. This is con-

<sup>24</sup> Compt. Rend. Soc. Biol., 1921 (84), 606.

firmatory of the impression that glycerol in some manner furnishes the bacilli the building material for the synthesis of its wax.

The proportion of total tubercle bacillus lipin present as wax has been determined by Long and Campbell.<sup>25</sup> Tubercle bacilli of human and bovine types were dehydrated with cold alcohol, boiled in hot absolute alcohol and subsequently leached with medium boiling petroleum ether. The hot alcohol extract was evaporated to dryness and the residue extracted with petroleum ether, this extract being added to the other petroleum ether extract. A fraction over 22 per cent of the dry weight of the bacillus was found in the case of each type to be soluble in petroleum ether. The lipin removed was treated five hours with boiling 25 per cent alcoholic potash. The saponification numbers for human type and bovine type bacillus lipins were respectively 131 and 139. The non-saponifiable lipoid, that is to say the waxy material which previous investigators had found to be a very difficultly hydrolyzable ester, probably a lauric acid ester of an alcohol of high molecular weight, was found to represent 77.1 per cent of the total lipin of the human type bacillus, and 60 per cent of that of the bovine type bacillus. The bulk of the lipin of the tubercle bacillus is thus waxy in nature. A smaller proportion of wax, as disclosed by this investigation, exists in the lipin of other types of acid-fast bacilli (see chapter on other acid-fast bacilli).

Long<sup>26</sup> has extended the observations of Aronson, Bulloch and Macleod and others on the difficult extractability of part of the lipin of tubercle bacilli, finding that from bacilli thoroughly defatted by the method just outlined, from 4 to 8 per cent of the dry weight of the bacillus could still be obtained in the form of a fatty material, by treatment of the defatted bacilli with normal hydrochloric acid at 38°, dehydrating with alcohol, drying and reextracting with petroleum ether. The lipoid product appeared to be the same wax as that which makes up the bulk of the total lipin (see Chapter III on acid-fastness).

<sup>25</sup> Amer. Rev. Tuberc., 1922 (6), 636.

<sup>26</sup> Amer. Review Tuberc., 1922 (6), 642.

## PROTEINS

In comparison with the expenditure of effort upon the lipins of the tubercle bacillus, its proteins have been much less extensively investigated. This is of course an anomalous situation in view of the fact that the biological individuality of the organism and the specificity of the immune response to its presence within the host, are presumably to be correlated with its protein content. The greater part of the work which has been done antedates modern classification, and modern methods have not been used to any great extent in the separation of the proteins present. Two points which have been at issue so far are the total quantity of protein in the bacillary protoplasm and the extent to which it is conjugated. Experiments on the toxicity of the protein and its relation to the phenomenon of sensitization, have been carried out on a wide scale, but their correlation with chemical composition is so remote as to leave them out of the scope of this book. For observations of this nature the reader is referred to other works, especially those on immunity. Some consideration of the field of immunity as seen from a chemical point of view will, however, be given in the chapter to follow, on tuberculin.

Hammerschlag,<sup>27</sup> while chiefly concerned with the lipins of the tubercle bacillus, attached much importance also to its protein constituents, to which he, unlike the majority of later observers, attributed the organism's acid-fastness, taking that stand because of the fact that the most complete lipin extracts he was able to make, left the bacilli still resistant to acid decolorization. In his original experiments he established the presence of a considerable amount of protein by taking up the alcohol-ether-insoluble residue in 1 per cent potassium hydroxide, extracting ten hours in the cold and one hour on the water bath, and decanting. The alkali extract was precipitated with ammonium sulphate. The precipitate gave the biuret, xanthoproteic and Millon reactions.

Weyl<sup>28</sup> extracted six hundred tube cultures scraped from glycerol agar, with warm sodium hydrate solution. The cloudy,

<sup>27</sup> Zent. klin. Med., 1891 (12), 9. Through Jahresbericht. f. d. Tierchemie, 1891 (21), 481.

<sup>28</sup> Deut. med. Woch., 1891 (17), 256.

yellow extract gelatinized on cooling. The jelly was taken up in warm water and filtered, leaving a considerable residue which gave protein reactions only doubtfully positive. Dilute acids produced in the filtrate a flocculent precipitate insoluble in dilute acetic acid. The product was purified by repeated solution in sodium hydrate, precipitation with acetic acid, and filtration. Ultimately a white product was obtained, which was washed with alcohol and ether and dried to a crumbly, white mass over sulphuric acid *in vacuo* and then at 105°. Combustion analysis gave the following figures: C, 51.6 per cent; H, 7.3 per cent; N, 4.4 per cent; sulphur and phosphorus were present, but the amounts were not determined.

This material was not affected by pepsin-HCl. Because of this fact and its insolubility in an excess of acetic acid it was thought to resemble the mucins most closely. However no reducing substance was split off on several hours boiling with 3 per cent sulphuric acid. This did not disprove the idea of a mucin, in Weyl's mind, for the amount subjected to analysis was small, and, as he points out, not all mucins split off a sugar on hydrolysis. In the light of modern knowledge, however, in view of the phosphorus content of the substance, its difficult solubility in an excess of acetic acid, and its resistance to pepsin digestion, we have reason for believing that the substance with which Weyl was dealing was a nucleoprotein.

Klebs<sup>8</sup> later definitely characterized the bulk of the substance as nuclein, on the basis of the resistance of the fat-free material to pepsin-HCl digestion, its solubility in alkali, precipitation by alcohol and content of phosphorus of 8 to 9 per cent, all of them corresponding to Miescher's findings for the nuclein of salmon sperm.

Hofmann<sup>29</sup> made water, acid and alkali extractions of the tubercle bacilli obtained from 42 glycerol-agar cultures. First he extracted the mass for eight days in the ice box with distilled water. The extract, filtered through kieselguhr, gave the biuret and xanthoproteic reactions. It was precipitated by addition of alcohol to a concentration of 60 per cent, as a white flocculent mass which gave protein reactions. This water-soluble substance

<sup>29</sup> Wien. klin. Woch., 1894 (7), 712.

was considered an albumin. Extraction of the residue remaining on the kieselguhr, with 1 per cent hydrochloric acid, and passage again through kieselguhr, yielded a filtrate giving protein reactions, in which no precipitate occurred on neutralization with sodium bicarbonate. Addition of alcohol, however, brought down a substance giving protein reactions. From its insolubility in water and solubility in the sodium chloride solution produced on neutralization of the hydrochloric acid extract by sodium bicarbonate, it was considered a globulin.

The amounts of albumin and globulin thus secured were small. A larger yield of protein was obtained on extraction with 0.2 per cent potassium hydroxide and precipitation with hydrochloric acid. This was considered to be of the nature of acid albumin. It dissolved readily in dilute sodium hydroxide solution, and coagulated at 60°. Alkali soluble protein, not precipitable by hydrochloric acid, remaining in the original alkaline extract, came out on addition of alcohol. Hofmann laid stress on the fact that the protein substances thus removed from the tubercle bacillus by alkaline extraction, affected healthy and tuberculous guinea pigs, respectively, in exactly the same manner as tuberculin, as small an amount as 0.0009 gram being sufficient to elicit reaction in a tuberculous pig, while healthy pigs did not give a reaction until the dose reached 0.0035 gram.

A more ambitious study of the proteins was that of Ruppel<sup>30</sup> who investigated the chemical composition of 255 grams of desiccator-dried tubercle bacilli coming from 50 liters of glycerol bouillon. He noted, like Weyl, that extraction of intact bacilli with alkali yielded a slimy liquid, giving a phosphorus-containing precipitate with acetic acid and alcohol. This he considered nucleoprotein, not mucin, as Weyl had believed it to be. He supplemented Weyl's observations, moreover, with one which the latter failed to make, namely, that extraction with alkali removed a reducing substance. Reducing substances will be considered in the section to follow on carbohydrates. The total amount of substance extracted from tubercle bacilli by dilute alkali Ruppel found to be about 15 per cent.

By various methods of extraction much larger yields of protein substances were obtained, the largest, 31.7 per cent resulting from

<sup>30</sup> Zeit. physiol. Chem., 1898 (26), 218.

preliminary extraction with 1 per cent soda solution, followed by drying, defatting, and then by two hours autoclaving in 5 per cent glycerol at 150°. The latter treatment alone took out 18 to 20 per cent, consisting exclusively of "albumose-like products, entirely analogous to the amidalbumoses of Neumeister." Such an experiment, while giving information concerning the amount of protein present in the bacillus, of course yields little insight into the original state of that protein, because of the undoubtedly digestive action of the autoclaving.

The alkali, alcohol-ether and hot glycerol-water extracts together amounted to 55.2 per cent of the original dry weight. The washed residue was a gray mass giving protein reactions. Solution of the greater part of this was accomplished by treatment with hot, concentrated hydrochloric acid. The solution thus obtained readily reduced cupric oxide in alkaline solution, a finding which suggested to Ruppel that the residue contained keratin or chitin, and that it was because of the presence of these difficultly soluble substances that the residue was so resistant to extraction.

Ruppel made a more interesting observation following an entirely different method of extraction. Robert Koch had found that dry bacilli might be so finely ground that none remained intact and that from the resultant powder about half the substance could be removed by simple trituration with water. A milky extract resulted, which could be freed from the larger particles by centrifugation. Taking advantage of this method Ruppel confirmed the observation that almost half of the powder went into solution. A yellowish, slightly opalescent, slightly alkaline or neutral extract resulted. This contained no coagulable protein and gave of all the protein color reactions only a feeble biuret test. Its most conspicuous property was that of precipitating true proteins from their solutions. The substance responsible for this property could be precipitated from the solution by the addition of acetic acid, the precipitate not being soluble in excess acid. It contained 4 per cent phosphorus, and gave the biuret test, but not the Millon or xanthoproteic tests. From 25 grams of this material 1 per cent sulphuric acid removed a substance, 0.7 gram in weight, precipitated as a white flocculent mass by alcohol.

This precipitate when treated with barium hydrate to free it from the sulphate entering into its composition, and leave the free base, yielded an alcohol-insoluble product, soluble in water, giving an alkaline reaction. An insoluble picrate also was prepared by the addition of sodium picrate to the sulphuric acid solution. The picric acid in turn could be removed with ether. The substance obtained in this way was phosphorus-free and gave only the biuret of all the protein reactions. In ammoniacal solution it precipitated true proteins and primary albumoses from their solutions. It thus fulfilled all of Kossel's criteria for protamines. Ruppel called it "tuberculosamine."

This protamine was in the original state bound to an acid which contained phosphorus. This acid could be obtained from the residue left after sulphuric acid extraction of the 25 grams of material referred to above. It possessed all the properties of nucleic acid. To the presence of a certain amount of this substance in the free state was attributed the precipitating power for proteins of the original bacillary extract. This free acid was obtained by treating the filtrate from the acetic acid precipitation, with alcohol and hydrochloric acid. The product, after washing with alcohol and ether, dried to a white powder, soluble in dilute alkali, giving none of the protein reactions, precipitating proteins from their acid solutions, and containing 9.42 per cent phosphorus. This agrees well with more recent analyses of nucleic acids from various sources which have a phosphorus content between 8 to 10 per cent. Ruppel called this acid "tuberculinic acid." The finding of an excess of this protein-precipitating substance in the extract of ground bacilli, explained why no coagulable protein was found in that solution.

In a later report Ruppel<sup>31</sup> gives this quantitative analysis of tubercle bacilli:

|                            | grams |
|----------------------------|-------|
| Tuberculinic acid.....     | 8.5   |
| Nucleoprotamine.....       | 24.5  |
| Nucleoprotein.....         | 23.0  |
| Fat and wax.....           | 26.5  |
| Mineral substances.....    | 9.2   |
| Proteinoid substances..... | 8.3   |
|                            | <hr/> |
|                            | 100.0 |

<sup>31</sup> Beitr. z. exp. Therap., 1900 (4), 87.

The tuberculinic acid, which uniformly contained 9.2 to 9.4 per cent phosphorus, gave on hydrolysis a "phosphorus-containing acid, tuberculo-thymic acid," and a considerable amount of guanine and small amounts of adenine and xanthine.

Behring<sup>32</sup> on the basis of Ruppel's work and his own in collaboration with Kitashima, came to the conclusion that the toxicity of the tubercle bacillus, and likewise of tuberculin, was due to their content of tuberculinic acid and specifically to the "tuberculo-thymic acid entering into its composition," an idea which has been considerably modified since their time.

Almost contemporaneously with Ruppel's work, and in the years following, Levene<sup>33</sup> made several studies on the nucleoproteins and nucleic acid of the tubercle bacillus. In his first communication he stated that three different protein substances could be extracted from tubercle bacilli, the first, having a coagulation range of 56 to 64°, being precipitated by 50 to 85 per cent concentration of magnesium sulphate, the second, coagulating between 72 to 75°, by full saturation with magnesium sulphate, and the third, coagulating at 94.5°, by complete saturation with ammonium sulphate. Saturation with ammonium chloride precipitated the first of these. All of these substances contained phosphorus and were thus probably nucleoproteins.

In the second investigation comparison was made of the products of growth on different media. Bacilli were cultivated on glycerol-peptone broth and on one of Proskauer and Beck's media, containing glycerol, potassium acid-phosphate, magnesium phosphate, ammonium sulphate and mannite. The product of growth, well washed, was dried *in vacuo* and then at 105°. Analysis of the products from the two media gave the tabulated figures on page 23.

The higher percentages of carbon, hydrogen, nitrogen and sulphur, would seem to indicate a higher protein content in the fat-free broth bacilli than in the others. The lower phosphorus content might mean a smaller nuclein content, or it might be correlated with the high ash.

Levene corroborated Ruppel's statement that the powdered bacilli contained free nucleic acid, by extracting this acid with

<sup>32</sup> Berl. klin. Woch., 1899 (36), 537.

<sup>33</sup> N. Y. Med. Record, Dec. 17, 1898; Jour. Med. Res., 1901 (6), 135; 1904 (12), 251.

|          | BEEF BROTH<br>CULTURES | MANNITE<br>SYNTHETIC MEDIA<br>CULTURES |
|----------|------------------------|--|
|          | per cent               | per cent                               |
| Fat..... | 31.56                  | 22.18                                  |
| Residue: |                        |  |
| Ash..... | 5.92                   | 10.00                                  |
| C.....   | 55.58                  | 47.41                                  |
| H.....   | 8.46                   | 7.05                                   |
| N.....   | 9.39                   | 7.91                                   |
| S.....   | 1.39                   | 0.25                                   |
| P.....   | 0.59                   | 2.67                                   |

a 5 per cent sodium chloride and 8 per cent ammonium chloride solution. Picric acid and acetic acid were added to remove other proteins and from the filtrate the nucleic acid was obtained as the copper salt by the addition of cupric chloride. The precipitate was washed with water and alcohol repeatedly until copper and chlorine free, then with ether, and dried *in vacuo*. The combined nucleic acid of the bacilli was obtained by the usual method of alkaline hydrolysis of the previously extracted material in the presence of sodium acetate, and precipitation with alcohol. The precipitate was biuret-free and possessed all the properties of nucleic acids. Several products were prepared in this manner and analyzed. A great variability in composition, especially in the phosphorus content, was encountered, depending presumably upon the state of purity of the preparations. The averages for several of these products are as follows:

|                       | C     | H    | N     | P     |
|-----------------------|-------|------|-------|-------|
| Broth cultures.....   | 37.08 | 5.69 | 11.51 | 10.84 |
| Mannite cultures..... | 35.66 | 5.83 | 9.70  | 10.44 |

It must be recognized that these preparations were made at a time when the methods for the isolation of nucleic acid were far from perfect.

In the last piece of work of this series an attempt was made to isolate some of the bases characteristic of nucleic acid. Inasmuch as Ruppel, Behring and Kitashima had made the statement that thymine was one of the decomposition products of tubercle

bacilli, the search was especially directed toward this constituent of nucleic acid. The quest was one of considerable theoretical interest, inasmuch as the pyrimidine base thymine was then, as it still is, considered characteristic of animal as distinguished from plant nucleic acids. Using the Kossel-Jones method for the separation of pyrimidines, Levene obtained, from fat-free bacilli which had been hydrolyzed three hours with 25 per cent sulphuric acid in the autoclave at 150 to 175°, a mixture of two types of crystals which had the appearance under the microscope of thymine and uracil. Analysis showed this mixture to contain 24.04 per cent of nitrogen. As thymine contains 22.22 per cent nitrogen and uracil 25.05 per cent, Levene felt that the analysis verified his microscopic diagnosis of these two pyrimidine bases. From the mother liquor of these crystals, sulphuric acid was removed by baryta and a substance precipitated with picric acid. This was in turn decomposed with sulphuric acid and ether and the solution concentrated. Some crystals morphologically similar to cytosine sulphate, insufficient for analysis, separated out.

Later research has failed to confirm the presence of uracil. A very accurate investigation of the pyrimidines, in which the modern methods of T. B. Johnson and Baudisch were used, has been made by E. B. Brown.<sup>34</sup> Thymine and cytosine were definitely isolated in crystalline form from the nucleic acid obtained from 1 kgm. of dry bacilli. Uracil was not present. From the results of this investigation we are warranted in believing that tubercle bacillus nucleic acid is of the animal and not the plant type. Brown found also that there was more phosphorus present in defatted bacilli outside of the nucleic acid than in it, an observation indicating that estimations of the nucleic acid content of tubercle bacilli based on the phosphorus content of the fat-free material, are highly inaccurate. The nucleic acid isolated by Brown made up from 1 per cent to 1.5 per cent of the dry weight of the bacilli, a value lower even than the low value, 2 per cent to 3.5 per cent, calculated by Long on the basis of the purine content (see below). Ruppel, it will be recalled, isolated what he considered a nucleic acid and called "tuberculinic acid," which made up 8.5 per cent of the dry weight.

<sup>34</sup> Proc. Nat. Acad. Sci., 1922 (8), 187.

Other members of the group of nuclein bases have been identified. Tamura,<sup>26</sup> whose investigation on the lipoids has been given in detail and whose work on the proteins will be recorded below, obtained in the purine base fraction of the sulphuric acid hydrolysate of 70 grams of dry tubercle bacilli, a picrate melting at 281°, the base from which furnished a silver salt containing 28.97 per cent nitrogen. As adenine picrate melts at 280° and silver adenine contains 28.45 per cent nitrogen, the presence of this purine base was considered established. On long standing the solution from which adenine picrate had precipitated deposited a few crystals, too small in amount for further examination, which had the crystal form of hypoxanthine picrate. Guanine could not be identified in the purine fraction at all either by color reaction or precipitation with ammonia, nor could the presence of xanthine be detected.

Long<sup>25</sup> compared quantitatively the purine bases from human type tubercle bacilli grown on glycerol broth and on a synthetic medium containing ammonium chloride, glycerol, sodium and potassium phosphate, sodium chloride and magnesium sulphate. He isolated both guanine and adenine, identifying the former by the nitric acid color test for guanine and by the characteristic crystalline form of its hydrochloric acid salt, and the latter by its picrate melting at 280°. No trace of xanthine or hypoxanthine could be detected. His figures were as follows:

|   | GUANINE |          | ADENINE |          |
|---|---------|----------|---------|----------|
|   | Grams   | Per cent | Grams   | Per cent |
| Broth bacilli, 100 grams.....                         | 0.213   | 0.21     | 0.200   | 0.20     |
| NH <sub>4</sub> Cl-glycerol bacilli, 3.09 grams ..... | 0.012   | 0.39     | 0.009   | 0.30     |

Using the Levene and Jacobs<sup>26</sup> formula for nucleic acid Long calculated the total nucleic acid content of the dry bacillus from the values for adenine and guanine obtained. His figures were 2 per cent in the case of the broth-grown organisms and 3.5 per

<sup>25</sup> Amer. Review Tuberc., 1921 (4), 842.

<sup>26</sup> Jour. Biol. Chem., 1912 (12), 411.

cent in that of the bacilli grown with ammonium chloride and glycerol as sole source of nitrogen and carbon. He called attention to the fact that these figures indicated a smaller nucleic acid content than had previously been supposed to be present.

A study of the nucleic acid in the colon bacillus has been made by Schaffer, Folkoff and Bayne-Jones,<sup>37</sup> who used a method that has been found suitable for isolation of nucleic acid in yeast, but does not lead to the isolation of nucleic acid from animal tissue. The product thus obtained contains about the amount of phosphorus required for plant nucleic acid, half of which is easily split and half firmly bound. It contains guanine but no pentose so that it does not correspond fully to plant nucleic acids. They have not studied the tubercle bacillus by this method, and in this organism Bendix<sup>44</sup> believed that he had demonstrated the presence of pentose.

Returning to the proteins of the bacillus we find that several investigators have confirmed the developing impression that a considerable portion of the bacillary protoplasm is in the form of nucleoprotein. Auclair and Paris<sup>38</sup> in their series on the "poisons of the tubercle bacillus" prepared a nucleoprotein which they designated as "bacillo-casein," a para-nucleo-albumin, by heating defatted bacilli one hour at 80° in concentrated acetic acid until all dissolved. Sodium hydrate added until the solution was only faintly acid brought down a protein precipitate, which was used in certain experiments on toxicity and immunization.

Tamura<sup>20</sup> has made the most detailed examination on record of the cleavage products of the protein of the tubercle bacillus; fifty-four grams of defatted bacilli, corresponding to 70 grams of intact organisms, were triturated as described above with 66.6 per cent sulphuric acid. The triturate on dilution with water deposited a protein precipitate. It was in the filtrate from this, which was biuret-free, that adenine was discovered. It did not contain Ruppel's protamine. The precipitate, weighing 29.2 grams, gave all the protein reactions except those depending on the

<sup>37</sup> Johns Hop. Hosp. Bull., 1922 (33), 151.

<sup>38</sup> Arch. d. méd. exp., 1907 (19), 129; (20), 737; Compt. Rend. Acad. Sci., 1908 (146), 301.

presence of sulphur. The nitrogen content was 9.2 per cent and the phosphorus, 0.7 per cent. It was insoluble in water, salt solution or dilute acid, but soluble in weak alkali and concentrated sulphuric acid. This material on hydrolysis with 25 per cent sulphuric acid for fourteen hours, at the end of which time the biuret test was negative, was worked up by the method of Kossel and Kutscher.<sup>39</sup> Tables 1 and 2, taken from Tamura's article verbatim, record his results, excluding 6 grams of melanin. The high content of phenylalanine and valine is conspicuous.

Goris,<sup>41</sup> whose valuable work on lipins has been reviewed above, obtained by alcohol precipitation of the water from the lipin-free bacilli, a protein, which was perhaps partially nucleo-

TABLE 1

|                      | IN 28 GRAMS<br>PROTEIN<br>OF THE<br>TUBERCLE<br>BACILLUS | IN 40 GRAMS<br>PROTEIN<br>OF THE<br>MYCO-BACT.<br>LACT. | PROTEIN                       |                                     |
|----------------------|--|---|-------------------------------|-------------------------------------|
|                      |  |   | Of the tuber-<br>cle bacillus | Of the <i>Myco-<br/>bact. lact.</i> |
| Arginine.....        | 0.6049   | 1.1700  | 2.16                          | 2.927                               |
| Histidine.....       | 0.1426   | 0.1092  | 0.51                          | 0.27                                |
| Lysine.....          | 0.2057   | 0.1902  | 0.735                         | 0.475                               |
| Ammonia.....         | 0.0272   | 0.0358  | 0.097                         | 0.089                               |
| l-phenylalanine..... | 2.2000   | 2.2320  | 7.85                          | 5.58                                |
| l-proline.....       | 0.6500   | 2.0200  | 2.32                          | 5.05                                |
| Valine.....          | 2.6700   | 0.2580  | 9.35                          | 0.645                               |

protein as it contained 1.2 per cent phosphorus. This material on hydrolysis with hydrochloric acid and treatment with phloroglucine gave a precipitate indicating the presence of a hexose in the protein molecule. The aqueous solution of this protein was precipitated by phosphotungstic and picric acids, and the reagents of Tamet, Courtonne and Patein, and in the presence of hydrochloric acid by silicotungstic acid and the reagents of Valser and Bouchardat. It was readily thrown down also by 10 per cent hydrochloric or nitric acids and by trichloracetic acid. Millon's

<sup>39</sup> Zeit. physiol. Chem., 1900 (31), 165, as modified by Weiss,<sup>40</sup> for its amino acid content, after filtration.

<sup>40</sup> Zeit. physiol. Chem., 1907 (52), 107.

<sup>41</sup> Ann. Inst. Pasteur, 1920 (34), 1907.

reaction was positive, but curiously enough the biuret test was negative. The substance was active as a tuberculin, 0.025 gram killing tuberculous guinea pigs.

The alcoholic solution left after filtration from the precipitated substance just described, contained amino acids, among which leucine and tyrosine were identified.

TABLE 2

|  | IN 28<br>GRAMS<br>PRO-<br>TEIN<br>OF THE<br>TUBER-<br>CLE<br>BACIL-<br>LUS | IN 40<br>GRAMS<br>PROTEIN<br>OF THE<br>MYCO-<br>BACT.<br>LACT. | RECKONED FOR                |  |                              |  |
|--|--|--|-----------------------------|--|------------------------------|--|
|  |  |  | 100 grams<br>protein of the |  | 100 grams<br>nitrogen of the |  |
|  |  |  | Tuber-<br>cle<br>bacillus   | <i>Myco-</i><br><i>bact.</i><br><i>lact.</i> | Tuber-<br>cle<br>bacillus    | <i>Myco-</i><br><i>bact.</i><br><i>lact.</i> |
|  | grams  | grams  | grams                       | grams  | grams                        | grams  |
| Total nitrogen.....                      | 2.5640   | 3.2220   | 9.157                       | 8.090  | 100.0                        | 100.0  |
| A. Basic nitrogen.....                   | 0.2963   | 0.4823   | 1.085                       | 1.230  | 11.85                        | 15.21  |
| In arginine.....                         | 0.1963   | 0.3780   | 0.70                        | 0.946  | 7.64                         | 11.69  |
| In histidine.....                        | 0.0392   | 0.0350   | 0.14                        | 0.09   | 1.53                         | 1.11   |
| In lysine.....                           | 0.0385   | 0.0398   | 0.137                       | 0.10   | 1.49                         | 1.23   |
| In ammonia.....                          | 0.0224   | 0.0295   | 0.105                       | 0.07   | 1.15                         | 0.91   |
| B. Monoamino acid.....                   | 1.7087   | 2.1560   | 6.098                       | 5.400  | 66.59                        | 66.74  |
| In l-phenylalanine.....                  | 0.1881   | 0.1891   | 0.67                        | 0.475  | 7.31                         | 5.89   |
| In l-proline.....                        | 0.0792   | 0.2466   | 0.28                        | 0.62   | 3.09                         | 7.66   |
| In valine.....                           | 0.2945   | 0.0284   | 1.05                        | 0.07   | 11.50                        | 0.91   |
| Other monoamino acids.....               | 1.1469   | 1.6925   | 4.09                        | 4.25   | 44.98                        | 52.34  |
| C. N in unknown form.....                | 0.5590   | 0.5837   | 1.996                       | 1.464  | 21.80                        | 18.09  |
| Humin-N.....                             | 0.4050   | 0.4680   | 1.446                       | 1.175  | 15.79                        | 14.52  |
| In the silver precipitate ..             | 0.0657   | 0.1040   | 0.23                        | 0.260  | 2.56                         | 3.21   |
| In filtrate from lysine-<br>picrate..... | 0.0873   | 0.01165  | 0.31                        | 0.029  | 3.38                         | 0.36   |

## CARBOHYDRATES

The earliest investigators of the chemistry of the tubercle bacillus reported the presence of carbohydrates, or at least of substances which reduced cupric oxide in alkaline medium. No such systematic study of them, however, has been made as has been the case with fats and proteins. Scattered observations appear in many papers, most of them accidental discoveries made in the course of analysis for other constituents of the tubercle

bacillus. It is impossible to build these up into anything like a complete survey of the subject.

The problem is complicated by the fact that carbohydrates may be free or in chemical combination with the other two great organic divisions, as glycolipins and glycoproteins. As a matter of fact we have evidence that reducing substances are present in all these states. Indeed it would be cause for surprise if they were not, in view of the wide distribution of such compounds in nature. Furthermore, the identification of nucleic acid carries with it the certainty of the presence of some sugar or other, sugar being an invariable constituent of nucleic acids, although variable in type.

The chief interest in the subject centers around the question as to whether or not there is a supporting frame work or enveloping membrane, comparable to what we have in plant cells or in the hard parts of insects. The search, never very vigorous, has waned in intensity in recent years, so that old reported findings of cellulose on the one hand and chitin on the other stand unrefuted and likewise unconfirmed.

Hammerschlag,<sup>27</sup> who made the first accurate studies of the chemical composition of the tubercle bacillus, found that he had left after the extraction of lipins by fat solvents and proteins by potassium hydrate, a substance which after boiling with sulphuric acid reduced cupric salts. Simple boiling with water failed to liberate a reducing substance. A certain amount of the material appeared to be soluble in an ammoniacal solution of copper oxide (Schweitzer's reagent). These facts led their observer to conclude that the residue left after the removal of fat and protein was cellulose. Accordingly, Hammerschlag gave the following composition to the tubercle bacillus: Fatty substances, 27 per cent; protein, 37 per cent; cellulose, 28 per cent; ash, 8 per cent.

Freund in 1886, and Kabrhel and G. Lange and Dreyfuss<sup>42</sup> reported that cellulose was present in tuberculous organs, as caseous lymph glands and lungs, both in man and cattle, and not in normal organs. They believed it was present in the bodies of tubercle bacilli. Nishimura, who was unable to obtain cellulose from tubercle bacilli which had been cultivated in the laboratory,

<sup>42</sup> Zeit. physiol. Chem., 1894 (18), 358.

thought it might be formed under different nutritional conditions in the animal organism. Their claims may be consulted in the original, or in Jolle's chapter in Ott's "Chemical Pathology of Tuberculosis," and need not be considered longer here. Certainly in such heterogeneous mixtures tests for cellulose would be at the best exceedingly difficult and the results doubtful.

Ruppel<sup>30</sup> found that extraction of the bacillus with 1 per cent sodium hydrate yielded a difficultly filterable solution which reduced alkaline copper oxide, the reducing power being increased by heating the extract for some time with acids. This substance could not be precipitated from solution by addition of acetic acid (difference from true mucin) but came out on treatment with acetic acid plus alcohol.

Ruppel also noted that his "rest-bacilli," which had been first defatted and then extracted at 150° with glycerol water, losing 55 per cent all told of their substance, on hydrolysis with concentrated hydrochloric acid liberated substances reducing cupric oxide. This material, which would not go into solution on long treatment with dilute mineral acids, even at temperatures of 150 to 200° under pressure, and which Ruppel considered as the frame work of the organism, gave protein reactions and would not dissolve to any noticeable extent in Schweitzer's reagent. It was accordingly considered, not cellulose, but a "proteinoid substance containing carbohydrate, and analogous to keratin or chitin," the structure of which was then less fully understood than it is today.

On the basis of this speculation on the nature of the substance, for it was little more than that, and an accidental discovery of acid-fastness in *tænia larvæ*, Helbing<sup>43</sup> was led to conclude that chitin, or a substance like it, was responsible for the acid-fastness of tubercle bacilli. This work will be considered in the chapter on acid-fastness. It is only mentioned here because Helbing is frequently cited as an authority for the presence of chitin in the tubercle bacillus. There is no chemical basis for such a conclusion in his article.

Bendix,<sup>44</sup> his attention caught by Nishimura's and Galeotti's reports on the presence of nucleoproteins and nuclein bases in

<sup>30</sup> Deut. med. Woch., 1900 (26), 133 V.

<sup>44</sup> Deut. med. Woch., 1901 (27), 18 O. A.

various bacteria, searched for the presence of what he considered another characteristic of nucleins, namely pentose, in the tubercle bacillus. He noted first that bacilli hydrolyzed by heating with hydrochloric acid, liberated a reducing substance, and from the hydrolysate he secured on treatment with phenyl hydrazine an osazone melting at 153 to 155°, which was considered characteristic for pentosazones. The orcin hydrochloride test of the gray powder gave a green color. An amyl alcohol extract of such orcin-treated material gave absorption bands at the red end of the spectrum between C and D, which again was considered typical for pentoses. Finally he was able to identify the same material in a nucleoprotein which he prepared by Hammarsten's method from intact bacilli. All of these considerations convinced him that pentose was present in tubercle bacilli and specifically in the nucleoprotein portion of it.

Levene<sup>33</sup> found a glycogen-like body in bacilli cultivated on mannite media or on ordinary glycerol broth. This substance came out in the sodium or ammonium chloride extraction, and remained with the nucleic acid after the picric acid precipitation of proteins. It remained in solution in turn after the nucleic acid was precipitated as the copper salt, and could be precipitated itself with alcohol. Repeated solution in dilute hydrochloric acid and precipitation with alcohol sufficed to free it from copper. The material thus obtained gave an opalescent solution, which on the addition of iodine gave a color test similar to that with glycogen, and was precipitated by basic lead acetate. It did not reduce Fehling's solution directly but liberated reducing substance on acid hydrolysis. As prepared it contained traces only of nitrogen and phosphorus. No subsequent investigation of this interesting glycogen-like compound seems to have been made, unless it was the same as the "pectin" described by Panzer (see below).

Baudran<sup>15</sup> was another who believed that the tubercle bacillus contained cellulose. By macerating tubercle bacilli eight to ten days with 1 per cent hydrochloric acid, he obtained a residue containing much waxy material and also a substance which was considered to be cellulose, in view of its solubility in a hot mixture of equal parts of zinc chloride solution and hydrochloric acid (concentrations not given), and its characteristic color reaction

with the chlor-iodide of zinc. This substance was believed to be part of the membrane of the organism and to make up 3.6 to 5.5 per cent of its dry weight.

Panzer<sup>18</sup> obtained on extraction of tubercle bacilli with hot water a substance which in concentrated solution gelatinized on cooling, and resembled gum arabic. It contained no nitrogen, phosphorus or sulphur. It was precipitated by alcohol and by lead acetate, and reduced Fehling's solution after hydrolysis by hydrochloric acid, not before. The hydrolysate also gave the  $\alpha$ -naphthol reaction of Molisch. On oxidation with nitric acid, oxalic acid was produced, but no mucic acid (test for galactose). Panzer concluded that the extracted gel was a pectin which was not a galactan.

The residue left after hot water extraction was resistant to pepsin digestion, and on hydrolysis with hydrochloric acid reduced Fehling's solution. The author made no further analysis but believed the material might be chitin. There was of course little enough reason for thinking so, as the substance unquestionably contained nucleoprotein, and therefore the sugar molecule entered into its composition. Unfortunately Panzer's experiments, which were valuable for their application of the digitonin method in the examination of tubercle bacilli for cholesterol, were made on too small an amount of material to permit many conclusions on the composition of other fractions. It should be mentioned, however, that he noted that alcohol removed from the bacilli something which reduced Fehling's solution. In this case he was probably dealing with a glycolipin.

Kozniewski<sup>19</sup> attempted by more modern methods than had been used in the earlier studies of Ruppel, to learn if chitin really was present in tubercle bacilli. He noted that mineral acids in a concentration of 3 to 5 per cent set free an inactive reducing sugar. The source of this seemed to be polysaccharides and doubtless was, in part at least, the same as those described by Nishimura, Levene and Panzer. The application of Winterstein's method for the isolation of glucosamine failed to indicate the presence of chitin.

Agulhon and Frouin,<sup>22</sup> in a study of the fats of the bacillus, obtained a phosphatid which they believed belonged to the class

of jecorins, as it liberated on hydrolysis, besides fatty acid, glycerol, and a base analogous to choline, a gummy polysaccharide from which glucose split off on further hydrolysis.

## MINERAL CONSTITUENTS

The mineral composition of the tubercle bacillus probably varies more than any other portion and doubtless is considerably affected by the content of the medium in diffusible inorganic substances.

Cramer's report<sup>45</sup> on the fluctuation in ash content of *Sp. cholerae* with changes in the composition of the medium, probably represents the situation in other bacteria as well. He found that increasing the amount of sodium chloride in the medium more than doubled the percentage of chlorine in the germs, while lowering the percentage of SO<sub>4</sub> and P<sub>2</sub>O<sub>5</sub>. When sodium phosphate was added the P<sub>2</sub>O<sub>5</sub> of the ash was much increased while the chlorine was decreased.

That the mineral constitution is not, however, directly dependent upon the mineral composition of the medium, is shown by the following figures of Krauss and Siebert,<sup>46</sup> in which a comparison is made of the ash of broth-grown tubercle bacilli and a sample of the kind of broth on which they were grown:

|                        | ASH OF BROTH                           | ASH OF DRY<br>TUBERCLE BACILLI       |
|------------------------|--|--------------------------------------|
|                        | Ash = 28.26 per cent<br>of dry residue | Ash = 7.52 per cent<br>of dry weight |
|                        | Composition                            | Composition                          |
| Cl.....                | per cent<br>43.98                      | per cent<br>6.60                     |
| PO <sub>4</sub> .....  | 8.57                                   | 51.25                                |
| SO <sub>4</sub> .....  | 2.25                                   | 0.84                                 |
| SiO <sub>2</sub> ..... | 0.26                                   | 0.19                                 |
| Na.....                | 29.69                                  | 9.18                                 |
| K.....                 | 14.69                                  | 26.55                                |
| Mg.....                | 0.41                                   | 3.22                                 |
| Ca.....                | 0.15                                   | 2.17                                 |

<sup>45</sup> Arch. f. Hyg. 1897 (28), 1.

<sup>46</sup> Taken from Cornet and Kossel in Kolle and Wassermann's Handbuch der Pathogenen Mikroorganismen, 1913 (5), 431.

A noteworthy accumulation of phosphate and of magnesium and calcium has occurred. A considerable share of the phosphate is of course the result of the ashing of those organic compounds containing phosphoric acid, the phospholipins and nucleic acid, which have been built up at the expense of the phosphate of the medium, but in the case of calcium and magnesium a selective absorption of inorganic material seems to have occurred. The relatively low concentration of sodium chloride in the bacillus and relatively high content of potassium, remind one of the conditions obtaining within animal cells bathed by blood plasma.

Other results on the ash of tubercle bacilli are as follows. There is marked disagreement with respect to the content of certain metals and acid radicals, particularly of sulphate. Hammer-schlag<sup>47</sup> found that on ignition of fat-free bacilli 8 per cent remained as ash. This would correspond on his figures to 5.84 per cent of the dry intact bacilli. De Schweinitz and Dorset<sup>6</sup> compared the ash of tubercle bacilli quantitatively with that of *B. mallei* and *B. suipestifer*. Tubercle bacilli grown on a glycerol, asparagin and ammonium phosphate medium gave 1.92 per cent ash, while analyses for ash of tubercle bacilli grown on ordinary glycerol broth varied from 1.77 to 4.03 per cent. The figures for *B. mallei* and *B. suipestifer* cultivated on bouillon were respectively 5.18 per cent and 12.41 per cent. In a later communication they reported on analysis<sup>48</sup> of the ash of bacilli grown on beef broth containing 1 per cent peptone and 7 per cent glycerol. The bacilli were killed by heat, washed with hot water, dried over sulphuric acid and powdered, and then thoroughly extracted with ether and alcohol. After this they were again dried and ignited at a low red heat. The total ash available was 1.453 grams. The amount of bacillary substance used is not stated. Examination showed that sulphate and chloride were absent. The partition analysis of the material gave the following figures: Na<sub>2</sub>O, 13.62 per cent; K<sub>2</sub>O, 6.35 per cent; CaO, 12.64 per cent; MgO, 11.55 per cent; C+Si, 0.57 per cent; P<sub>2</sub>O<sub>5</sub>, 55.23 per cent.

The conspicuous findings in this analysis were the high content of phosphorus, calcium and magnesium, which came thus

<sup>47</sup> Monatshefte f. Chemie, 1889 (Through Ott).

<sup>48</sup> Cent. f. Bakt., 1898 (23), 993.

to be considered by De Schweinitz and Dorset as essential elements in the biochemistry of the tubercle bacillus. In a later piece of research<sup>49</sup> in which they found an ash figure varying from 2.31 to 3.96 per cent, they reported P<sub>2</sub>O<sub>5</sub> as high as 74.38 per cent.

Other figures of which many might be cited, vary from 2.55 per cent ash with Kresling, to 5.92 per cent for bacilli grown on broth and 10 per cent on a synthetic ammonium phosphate glycerol-mannite medium, with Levene.<sup>33</sup>

Goris and Liot<sup>23</sup> analyzed the ash from 33.12 grams of tubercle bacilli, obtaining 0.842 gram or 2.50 per cent; 32.1 per cent of this was left as calcium sulphate after extraction with dilute nitric acid. The acid-soluble mineral constitution was as follows: P<sub>2</sub>O<sub>5</sub>, 43.4 per cent; Na, 11.6 per cent; K, 7.7 per cent; Mg, 5.7 per cent; Ca, 9.7 per cent; SO<sub>4</sub>, 22.8 per cent.

Iron, manganese and zinc were detected in traces. An examination of the crude lipin of the bacillus was also made, in which 1.16 per cent of the mass remained as ash, which had a phosphate content corresponding to 5 per cent of lecithin in the fatty material.

#### RECAPITULATION

As seen in the compilation above, the tubercle bacillus consists, like all bacteria and cells in general, of the usual organic constituents of protoplasm, namely, lipins, proteins, carbohydrates and inorganic substances. It differs from the majority of other bacteria in its possession of a relatively large amount of lipin and perhaps also in the manner of disposition of this type of material within the cell body.

From the figures quoted we may conclude that approximately 40 per cent of the dry weight of the bacillus consists of fat-like substance;—substance, that is to say, extractable with the common fat solvents such as ether, chloroform, benzene, toluene and xylene. Evidence has been brought forth to show that the amount varies somewhat with the strain of bacillus, the bovine type being richer in lipin than the human type, and that it

<sup>49</sup> Twentieth Annual Report of the Bureau of Animal Industry of the U. S. Dept. of Agriculture.

varies also with the composition of the medium. Accepting Frouin's results on the relatively low fat content of bacilli grown on glycerol-free media we are perhaps warranted in believing that the reported analyses of the large crops of bacilli grown on glycerol broth do not represent accurately the condition of the parasite within the tissues of the animal body, where the concentration of glycerol is by no means so high as in the usual glycerol broth. Even if this be so, basing our notions on the lowest figures reported for tubercle bacilli grown upon media containing any glycerol at all, we must, however, conceive of this organism as distinguished from most other bacteria by its exceptionally high lipin content.

The lipin is, apparently, not true fat for the most part, but an ester, or mixture of esters, of the group of waxes. Because of the difficult saponifiability of this wax, reports on the saponification number of the "crude fat" of the bacillus have been rather inaccurate, but it is apparently in the neighborhood of 130, relatively low, that is to say, as compared with true fats, and an indication that the ester combination is one of compounds of high molecular weight. Prolonged alkaline hydrolysis liberates a waxy substance of the general group of sterols or solid alcohols. The closest approximation to purity in this substance has probably been attained by Tamura, who assigns the empirical formula  $C_{29}H_{55}OH$  to it on the basis of combustion analysis for carbon and hydrogen and on the amount of bromine and benzoic acid absorbed on bromination and benzoylation.

Much discussion has centered on the problem of the presence of cholesterol. The best analyses have failed to give any indication of its occurrence, either by the usual color tests or the digitonin precipitation method of Windaus. Furthermore, the melting points of the waxy residues after saponification differ greatly from that of cholesterol.

About 40 per cent of the total lipin, or approximately 16 per cent of the dry weight of the bacillus, consists of a solid alcohol, which is a relatively inert substance. The ester combination with various fatty acids, as it actually exists within the bacillus, makes up 60 per cent and upwards of the total lipin, or 15 to 25 per cent of the dry weight of the microorganism, and is also

relatively inert from the point of view of metabolic change. One is naturally led to speculate on the meaning of this storing of a non-labile substance. There is much value in Bürger's conception of its protective nature and his comparison with the similar conditions in the walls of xerophytic plants. Whether applied as a coating to the surface of the bacillus, or diffused uniformly through the protoplasmic mass, it must render the germ less permeable to destructive agencies and therefore more resistant to intracorporeal lysis. The track of development of this substance and its relation to animal pathology are lost in untold centuries of evolution of both host and parasite, but the protective rôle of the substance, the armoring that it has conferred in the age old struggle between man and his most destructive enemy, can hardly be doubted. The essential foreign-body nature of the host's response to the presence of the tubercle bacillus, which constitutes the pathological process tuberculosis, is unquestionably largely dependent upon the organism's high content of this non-labile material.

From all that may be gathered from the literature, true, neutral fats make up no great proportion of the total lipin of the bacillus. In fact the presence of glycerol, as indicated by the acrolein test, has been recorded but once or twice. On the other hand the presence of free fatty acids has several times been emphasized. If we may believe the results of Aronson, Kresling and Goris, the "fat" of the tubercle bacillus is already a rancid fat, containing about 15 per cent of its weight as free fatty acids. This is perhaps to be correlated with Ruppel's finding of a high free nucleic acid content also in its protoplasm.

Reliable quantitative results on the phosphatids are not at hand, but Tamura's work gives us some conception of their type. They seem to resemble lecithin in the general property of insolubility in acetone, but differ in that the ratio of phosphorus to nitrogen is 1 to 2, not 1 to 1. Tamura believed that they belonged to the diamino-monophosphatids. Modern methods will throw more light on this subject.

The bulk of the lipin is thus of the nature of wax, consisting largely of esters of alcohols of high molecular weight, chiefly one of the composition  $C_{29}H_{55}OH$ , with various fatty acids, apparently

largely lauric and palmitic acids. In addition to this there seems to be an appreciable amount of free fatty acid, a small amount of neutral fat, an undetermined but not large amount of phosphatid and a small, undetermined proportion of glycolipins. Substances of still less understood composition, like the "hyalinol" of Goris, are also present.

Published figures indicate that about half of the dry weight of the bacillus is of protein nature. The amino acid content has been studied by Tamura, who found nothing striking except a high content of phenyl alanine, and a not very high content of the diamino acids. The last result is difficult to reconcile with Ruppel's finding of a high protamine content in the bacillary protein, which would call for a correspondingly high content of constituent diamino acids, which, as is well known, are conspicuous in protamines.

A considerable portion of the protein is combined as nucleoproteins. The presence of the recognized basic constituents of nucleic acid has been recorded. Guanine, adenine, thymine, and cytosine have all been identified with certainty. In the finding of both thymine and cytosine, in the absence of uracil, the identification of the tubercle bacillus nucleic acid as of the animal type of nucleic acid, has been accomplished.

Albumins and globulins have been described, but the investigation of their nature has been far from complete. Proteins containing a sugar molecule are certainly present but have been little studied. All in all, work on the proteins of the bacillus is much in need of extension, especially in view of the fact that the manifestations of resistance and immunity are probably to be correlated with the protein composition of the bacillus.

Carbohydrates are known to be present in combination with both protein and lipoid and also in the free state. Whether the sugar molecules of the protein are confined to the nucleic acid of nucleoprotein or exist in other combination is not known. A pentose has been described by Bendix as a constituent of the nucleoprotein. A hexose-containing phosphatid of the class of jecorins has been reported among the lipins. In the earlier investigations much discussion centered on the presence or absence of cellulose in the enveloping membrane of the organism.

The problem has not been attacked by modern methods and old observations indicating the presence of cellulose or a similar carbohydrate stand unconfirmed. The suggestion that the nitrogenous carbohydrate chitin, might form part of the shell, has also been made, but specific search has failed to reveal the presence of its characteristic unit, glucosamine. Soluble polysaccharides, free from protein and fat, of a glycogen-like and gum-like character, have also been described.

There is little of exceptional note in the mineral constituents. The amount present depends to a certain extent upon the medium in which the organisms are grown and varies from 2 to 10 per cent. The usual figure is about 6 per cent. There is, however, a marked tendency to lay up phosphorus, which is of course to be correlated with the nucleic acid and phosphatid content of the bacillus. In addition a rather noteworthy accumulation of calcium and magnesium occurs. There is disagreement on the relative amounts of sodium and potassium in broth-grown bacilli. Chlorine is always present, sulphur may or may not occur, and iron, manganese and zinc have been detected in traces. A small amount of silica is usually found but perhaps is not a component of the bacilli themselves.

## CHAPTER II

### THE METABOLISM OF THE TUBERCLE BACILLUS

The tubercle bacillus, whether under cultivation in the laboratory or leading a parasitic existence in the animal body, must, like other cells, obtain its nourishment and excrete its waste products by the physical processes of diffusion and osmosis, modified as they may be by the character of the enveloping membrane or colloidal substrate of the microorganism. It multiplies in favorable media, not as rapidly as most other bacteria, and in so doing synthesizes in the course of time many times its original weight, of its peculiar lipins, proteins and carbohydrates, the composition of which has been considered in the foregoing chapter. To do this it must modify the constituents of its medium of growth in such a way as to render them available for synthesis. This process calls for certain tools of the class which we call enzymes, which will be considered in the pages to follow. The activities of the bacillus are of course not confined to synthesis, as its life processes, like those of any other cell, are accompanied by the expenditure of a certain amount of energy, which calls for the absorption of oxygen and the oxidation of some of its food materials. Furthermore, in the course of its metabolism certain organic compounds, apparently of no further use to the organism, may diffuse from its substance into the surrounding medium. In the text below, consideration will first be given to the enzymes with which the bacillus operates, then to the manner in which it obtains its nutrition, to the products used and the products excreted, and finally to the important metabolic products contained in "tuberculin."

#### ENZYMES

Inasmuch as the tubercle bacillus is distinguished chemically from other organisms chiefly by its higher fat content, it is not unnatural that among the few investigations on its enzymes the earliest should concern its lipases and esterases.

Wells and Corper,<sup>1</sup> who did the first systematic work on the subject, were able to find only one report prior to theirs, concerning the lipolytic enzymes in *B. tuberculosis*. Carrière<sup>2</sup> claimed that broth cultures of this organism cause acid formation from monobutyryl. The action was destroyed by heat, but not by chloroform or ammonium fluoride; hence he concluded that it must be due to a true lipase or butyrase. His evidence was, however, unconvincing, since the titrations were performed with sodium carbonate with phenol-phthalein as indicator, so that the quantitative value of his results is nothing. Also monobutyryl is not a conclusive indicator for the presence of fat splitting enzymes.

In their own investigation Wells and Corper used tubercle bacilli grown on glycerol agar and killed by twenty-four hours' treatment with toluene water. These were emulsified, and the emulsions divided, one-half being submerged one-half hour in boiling water to destroy all thermolabile enzymes. To each pair of samples was then added a portion of the ester to be tested, olive oil, ethylbutyrate or triacetin, a slight excess of toluene, and a few drops of phenol-phthalein solution. The mixture was made neutral, incubated, and titrated at intervals to determine developing acidity. Comparison was made with other bacteria at the same time. *B. tuberculosis* was shown to be, if not an active splitter of fats, at least capable of slowly hydrolyzing the three very different esters used in these experiments. It was less active than *Staphylococcus pyogenes aureus*, *B. pyocyanus*, *B. coli* and *B. dysenteriae*. Inasmuch as each bacterium exerted a corresponding effect upon each of the three sets of esters used, the authors concluded that probably the same enzyme attacked all three. As in the case of the lipases of mammalian tissues, the bacterial lipases were inhibited by sodium fluoride in high dilution.

Kendall, Day and Walker<sup>3</sup> verified this finding of a lipase in the bodies of tubercle bacilli, and amplified it, showing that bacilli of the human (rapidly growing, avirulent strain), bovine and avian types, as well as other acid-fast organisms, namely, the leprosy bacillus of Duval, smegma and grass bacilli, all formed an esterase

<sup>1</sup> Jour. Infect. Dis., 1912 (11), 388.

<sup>2</sup> Compt. Rend. Soc. Biol., 1905 (53), 320.

<sup>3</sup> Jour. Infect. Dis., 1914 (15), 443.

capable of splitting ethyl butyrate and castor oil. This enzyme was to be found in the medium of growth. A fact, difficult to understand, was that it was thermostable, resisting an exposure of fifteen minutes to 100° in the moist state without appreciable diminution of activity. The bodies of the bacteria freed from adherent media contained lipase also; this, however, did not seem to be in as high a concentration as that within the media. It was suggested that the enzyme was freed as a result of autolysis or excreted as an exolipase. It was found, too, that the lipase formed by a rapidly growing tubercle bacillus in the medium of growth was qualitatively the same, regardless of the composition of the medium, even when the latter consisted essentially of a solution of ammonium chloride, ethyl alcohol and sodium phosphate. The concentration of the lipase in the medium of growth appeared to be roughly proportionate to the luxuriance of the growth which had taken place in it. They noted also that lipolytic activity was extremely slight during the first day's growth of the organism and thereafter increased proportionately to active development of the bacilli. With cessation of development, and presumably some autolysis of bacilli, the lipolytic power of the medium, instead of increasing, as would be expected if the lipase were liberated as a result of autolysis, decreased. It therefore seemed to the authors that the organism secreted a soluble lipase.

Corper and Sweany<sup>4</sup> carried the investigation of the enzymes of the bacillus much farther by investigating its proteolytic as well as autolytic enzymes, and some other ferments which might play a rôle in its metabolism, urease, diastase and saccharase. In an organism initiating such widespread tissue destruction as occurs in tuberculosis it was a point of some importance to determine if it possessed enzymes capable of breaking down protein, particularly that kind of protein found in the elastic tissue of the lung. Modern methods of analysis were used on the problem with the following findings:

Tubercle bacilli of both human and bovine varieties possess autolytic enzymes, as indicated by the liberation of noncoagulable nitrogen from the bodies of toluene-killed bacilli kept at incubator

<sup>4</sup> Jour. Bact., 1918 (3), 129.

temperature. The bacilli themselves, or autolysates therefrom, also possess a trypsin-like enzyme capable of splitting proteins in alkaline solution, an erepsin-like enzyme capable of decomposing peptone in acid solution, a weak pepsin-like ferment splitting proteins in acid solution, a nuclease breaking down nucleic acid into soluble form, and a urease capable of decomposing urea. Diastases and sucrase could not be demonstrated; autolysates from tubercle bacilli did not have the capacity for digesting elastic tissue from lamb's lung, or the connective tissue from tubercles.

Other observations indicate that although tubercle bacilli undoubtedly do possess autolytic enzymes, these are not very active as compared with other cells or tissues. Long<sup>5</sup> found that 5 grams of moist tubercle bacilli killed by toluene and incubated two weeks liberated only 5 mgm. of soluble nitrogen.

Other enzymes, for the presence of which tests have been made, are reductases, oxidases and emulsin. In common with other bacteria, living tubercle bacilli have the property of reducing selenium and tellurium salts to the free metals (vital reaction of Gosio). Corper<sup>6</sup> found that if a small clump of living tubercle bacilli were placed in the cup of a hollow slide and one or two drops of sterile 0.2 per cent solution of sodium tellurite in distilled water added and the preparation incubated, blackening of the clump occurred in one-half to two hours. Belfanti<sup>7</sup> obtained the same result, using several varieties of tubercle bacilli. He noted at the same time that the organisms formed with the tellurium a synthetic compound with the garlic odor of tellurine. Reduction of methylene blue to the colorless form can also be observed by subjecting tubercle bacilli vitally stained with methylene blue to a few moments of anaerobic conditions.

The presence of catalase was demonstrated by Hahn,<sup>8</sup> who noted that juice expressed from tubercle bacilli under pressure decomposed hydrogen peroxide. This fact was confirmed by Long,<sup>9</sup>

<sup>5</sup> Amer. Review Tuberc., 1921 (4), 842.

<sup>6</sup> Jour. Infect. Dis., 1915 (16), 47.

<sup>7</sup> Zeit. f. Chemotherap. Orig., (1912) (1), 113.

<sup>8</sup> Münch. med. Woch., 1897 (14), 1344.

<sup>9</sup> Amer. Review Tuberc., 1919 (3), 86.

who found that the action of the catalase could be shown most simply by merely dropping a plaque of tubercle bacilli into a solution of hydrogen peroxide. A slow, steady evolution of gas takes place.

Goris<sup>10</sup> was unable to obtain any splitting of the glucoside amygdalin by preparations of the tubercle bacillus, nor did any of his preparations split poly- or disaccharides.

An interesting observation in the field of fermentations was that of Jobling and Peterson,<sup>11</sup> who found that the soaps of the unsaturated fatty acids of the tubercle bacillus, like other soaps of unsaturated fatty acids in their experience, had the power of inhibiting proteolysis. To this property they attributed the strikingly slow autolysis of the caseous tubercle.

#### NUTRITION

The tubercle bacillus is notoriously a slowly growing organism. This fact undoubtedly delayed its discovery and led to the publication of erroneous results on the isolation of the cause of tuberculosis, the secondary invaders of the disease process easily outgrowing the real agent. Koch<sup>12</sup> used coagulated blood serum, planting bits of tissue from tuberculous lungs and obtaining growth only after several weeks. This was the method generally used for the growth of the organism until Nocard and Roux<sup>13</sup> made the most important discovery ever made concerning the nutrition of the bacillus, namely, that glycerol as an ingredient of the medium was an invaluable aid to growth. Nocard came upon this interesting fact when he added glycerol as a hydrophilic agent to Koch's blood serum in order to prevent it from drying excessively during the long period required to obtain a satisfactory culture.

Since Koch's time the isolation of tubercle bacilli from sputum or from lesions, once considered very difficult, has become a simple matter. It is customary today to destroy other organisms in the material by treatment with alkali, as 2 per cent sodium hydrate, for one-half hour, to which the tubercle bacillus is almost

<sup>10</sup> Ann. Inst. Pasteur, 1920 (34), 497.

<sup>11</sup> Jour. Exp. Med., 1914 (19), 239; Zeit. Immunität., 1914 (23), 71.

<sup>12</sup> Berl. klin. Woch., 1882 (19), 221; Mitt. a. d. kaiserl. Gesund., 1884 (2), 1.

<sup>13</sup> Ann. Inst. Pasteur, 1887 (1), 19.

uniquely resistant, and plant the product upon a glycerol-egg medium enriched with peptone and meat extract, such as the medium described by Petroff.<sup>14</sup> After the first culture has been obtained, as has long been known, the bacillus will grow with fair luxuriance on glycerol peptone agar or glycerol-peptone broth.

The manner in which the various ingredients of the medium serve in promoting growth, and the changes which they undergo in the microorganism's metabolism, will be considered below.

#### NITROGEN METABOLISM

Like the great majority of bacteria, the tubercle bacillus obtains its nitrogen from the cleavage products of protein molecules, and, as will be brought out, makes ready use of the very simplest of these, viz., amino acids, acid amides, and ammonia. It is not itself proteolytic, in the sense that gelatin and casein liquefiers are, as its growth on coagulated egg media proves to the most superficial observation; but there are indications that it does use this protein storehouse gradually, when sufficient of the more readily absorbed food material is present in addition.

Bouveault<sup>15</sup> was apparently the first to make detailed study of the nitrogen utilization. On analysis of glycerol broth before and after culture of the tubercle bacillus, he found that ammonia and amino nitrogen were readily used, that creatine and creatinine were utilized, but that gelatin and peptone were left practically untouched. His finding of the ready utilization of ammonia was a confirmation of the already prevalent notion that bacteria had great powers of synthesis and in their assimilation of ammonia nitrogen behaved like the recognized members of the vegetable world.

A great step forward in an understanding of the physiology of the tubercle bacillus was taken by W. Kühne,<sup>16</sup> who noted that it would grow on a relatively simple medium of known composition. The following was found to permit moderately luxuriant, if slow, growth: Leucine, 4.0 grams; asparagine, 2.0; taurine, 0.5; ammonium mucinate, 2.0; glycerol, 40.0; sodium chloride, 5.0;

<sup>14</sup> See also Dorset, Amer. Med., 1902 (3), 555.

<sup>15</sup> Thèse de Paris, 1892.

<sup>16</sup> Zeit. f. Biol. 1894 (30), 221.

ash of 10 grams beef extract; water, 1000 cc. Multiplication on this medium afforded a clear demonstration of the ability of the micro-organism to synthesize its entire body substance from simple compounds.

Kühne apparently did not continue the lead, but his paper was followed shortly by the most exhaustive study ever made of the nutrition of this microorganism, or perhaps any microorganism. Proskauer and Beck,<sup>17</sup> starting with the Kühne formula, determined, by a series of omissions and substitutions, which were the growth-promoting constituents. Their preliminary observations indicated that leucine and asparagine were good sources of nitrogen, that alanine was as good as leucine, and that alanine in combination with asparagine was superior to alanine plus tyrosine, the latter amino acid being apparently inhibitory. The addition of taurine was distinctly detrimental.

Further observation brought out the fact that the amino acid glycine,  $\text{CH}_2\text{NH}_2\text{COOH}$ , was suitable as the sole source of nitrogen, while substituted glycines, like sarcosine,  $\text{CH}_2\text{NH}(\text{CH}_3)\text{COOH}$ , and tri-methyl-glycine, or betaine, were not suited for growth. Glycine combined with benzoic acid in hippuric acid did not support growth.

A great variety of other substances were tried as sources of nitrogen, combined with a suitable source of carbon, like glycerol. Glucosamine in 0.2 per cent concentration supported growth. Uric acid, alloxan, alloxanthine, allantoin, caffeine, guanine, urea and imido urea, could not be utilized. In general it was apparent from their work that ammonia and the simpler amino acids and acid amides were utilizable as sole sources of nitrogen, while substituted amino nitrogen and imino nitrogen were not; and that nitrogen in many of the more complex compounds, like the aromatic amino acids and purine bases and their derivatives, was not available, perhaps because of the inhibitory effect of the rest of the molecule. Nitrate nitrogen was unavailable and nitrites were distinctly toxic.

Proskauer and Beck's simplest formula which afforded growth, viz., ammonium carbonate, 0.35 per cent; mono-potassium

<sup>17</sup> Zeit. f. Hyg., 1894 (18), 128.

phosphate, 0.15 per cent; magnesium sulphate, 0.25 per cent; glycerol, 1.50 per cent; has been repeatedly quoted as producing bacilli reduced to the lowest terms. It is of course in effect a solution of ammonium phosphate, magnesium sulphate and glycerol. In my experience it has been found too alkaline to permit growth, although Proskauer and Beck stated that flasks containing such media became covered with a thin skin of tubercle bacilli eight weeks after inoculation. That ammonium phosphate and glycerol and certain inorganic salts in solution at the right reaction, are fully capable, however, of supporting growth, has been repeatedly demonstrated since.

Proskauer and Beck's work was simply a determination of the availability of certain compounds for the nutrition of the bacillus. They did not make extensive application of the facts learned to the more common conditions of bacterial growth as seen in complex media or in the actual condition of parasitism. Points obviously raised and left unsettled were these: If ammonia and amino acids were utilizable as sole sources of nitrogen, and if, as was also known to be true, ammonia and amino acids were themselves produced from protein by bacterial growth, was it not probable that it was in that form, chiefly, that protein became available for bacterial growth? Indeed, was it not likely that the amino acids themselves functioned largely as ammonia furnishers, inasmuch as a single amino acid sufficed for growth and therefore for the construction of all the amino acids in the bacterial protein? Since, however, growth was never as rapid on these so-called synthetic media as upon one in which the nitrogen was present in the form of peptone, was it not also probable that certain constituents of the peptone molecule, while capable of synthesis and therefore not absolutely necessary to maintenance and reproduction, were nevertheless distinct accelerators of growth as rather complex building blocks ready formed?

Tiffeneau and Marie,<sup>18</sup> whose work will be mentioned again below, among other investigations determined the amount of ammonia removed from the medium, using one of Proskauer and Beck's formulas, in which ammonium sulphate supplied all the nitrogen. Allowing their cultures to run over long periods, they found that

<sup>18</sup> Compt. Rend. Soc. Biol., 1912 (72), 48.

after eight months two-thirds of the ammonia had been removed from the medium to be incorporated in the bacillary substance of a growth product of 4 grams of dry organisms.

Kendall, Day and Walker,<sup>19</sup> using a rapidly growing tubercle bacillus of practically no virulence, found that ammonia accumulated rapidly at first in the course of culture in peptone-glycerol broth and then receded, the cause of the recession not being determined. The same two phenomena occurred in a medium in which asparagine served as the sole source of nitrogen. In general a parallelism was observed between the weight of the bacterial pellicle and the ammonia content of the broth. Thus the tubercle bacillus was to be considered an active deaminizer of the cleavage products of proteins. That it was at the same time capable of supporting life with ammonia as the sole source of nitrogen, was shown in a series of experiments in the same investigation, in which all the nitrogen present was in the form of ammonium phosphate. In fourteen days 10 per cent of the original content of the medium in this substance was removed by the growth of the bacillus. After twenty-eight days a slight amount returned, coincident with apparent autolysis of the bacilli.

Somewhat later Long<sup>9</sup> followed the course of ammonia liberation from a monoamino acid and an acid amide, glycine and acetamide, and from a single compound which contains both amino and amide nitrogen, asparagine. Ammonia was found to be liberated by the growth of the bacillus in all cases, but more rapidly from the amino group of glycine than from the amide group of acetamide. In asparagine the amide group was more readily attacked. The following table indicates the action of human and bovine type tubercle bacilli on this compound:

|                         | BEFORE<br>INOCULATION<br><i>mgm. per 100 cc.</i> | AFTER GROWTH<br>OF BOVINE<br>BACILLUS B <sub>1</sub><br><i>mgm. per 100 cc.</i> | AFTER GROWTH<br>OF HUMAN<br>BACILLUS H <sub>37</sub><br><i>mgm. per 100 cc.</i> |
|-------------------------|--|---|---|
| Amino N.....            | 57   | 46  | 54  |
| Amide N.....            | 56   | 11  | 10  |
| Ammonia N.....          | 2  | 35  | 28  |
| N used by bacillus..... |  | 23  | 23  |

<sup>19</sup> Jour. Infect. Dis., 1914 (15), 417.

Long also followed the nitrogen utilization in the growth of the same human type bacillus in glycerol peptone broth. His analyses gave the following figures:

|  | BEFORE<br>GROWTH | AFTER GROWTH<br>(ORIGINAL<br>VOLUME<br>RESTORED) |
|--|------------------|--|
|  | mgm. per 100 cc. | mgm. per 100 cc.                                 |
| Total N.....   | 133              | 61.0   |
| Amino N.....   | 24.3             | 22.0   |
| Ammonia N.....   | 5.0              | 10.0   |
| Other N by difference (imino N in proteose and<br>peptone) ..... | 103.7            | 29.0   |
| N used by bacillus.....  |                  | 72.0   |

In this experiment over half of the nitrogen originally present in the medium was converted into bacillary substance. The figures indicate a great reduction in the imino nitrogen of the medium, while the amino content remained nearly the same. This does not necessarily mean that peptone was used directly and free amino acids spared. Rather, in view of the known facts on amino acid utilization, it indicates that the peptone served as a storehouse for amino acids, the latter being produced from it as rapidly as needed, an approximately constant concentration being maintained.

Some doubt on the validity of this conclusion, however, was caused by the results of an experiment in which the rate of growth on pepsin digests and biuret-free trypsin digests of casein was compared. The growth on a laboratory broth containing commercial peptone and meat extract was noted at the same time for comparison. In all cases glycerol was present as a further and indispensable source of carbon, and phosphate was added.

Average dry weight of growth per flask after four weeks:

|                       | gram |
|-----------------------|------|
| Laboratory broth..... | 0.31 |
| Pepsin product.....   | 0.18 |
| Trypsin product.....  | 0.06 |

The growth on the pepsin digest, which must have consisted chiefly of peptone, was much superior to that on the trypsin digest,

which, being biuret-free, represented the free amino acids of casein. This result indicated that peptone was a better source of nutriment and that it might be used directly. However, it was believed by the author that this last was not the case, in view of the results of Rettger<sup>20</sup> and his associates, which indicated that amino-acid-free peptone was not directly available for bacterial nutrition, being utilized only when sufficient more readily assimilable nitrogen was present to construct the enzymic machinery for attacking the larger molecules. He cited the observation of Burroughs and Neyman<sup>21</sup> that amino acids, although available for direct anabolism by most cells, were nevertheless in a relatively low concentration directly toxic. It seemed probable to him that here again the peptone, which, being a crude pepsin product, contained an appreciable amount of free amino acids, served as a storehouse liberating the amino acids only as rapidly as needed.

Confirmation of the notion that it is the amino acids rather than the peptone which are directly used, has come from the work of Masucci,<sup>22</sup> who found it commercially profitable in the preparation of tuberculin to add a commercial preparation of free amino acids to the peptone in the broth used in the manufacture. Much larger yields of bacilli are thus secured.

The idea that amino acids are the essential nitrogenous nutrient of the tubercle bacillus and further that some of them are more valuable than others, has been developed chiefly by the French school. Armand-Delille, Mayer, Schaeffer and Terroine,<sup>23</sup> growing the bacillus on media of known composition, found that the addition of scombrine, the protamine from mackerel sperm, greatly increased growth. The favorable effect of scombrine, which is notable for its high content of the diamino acid arginine, was duplicated by substitution of arginine for it. The diamino acid histidine, although a growth promoter, was not so useful in this respect as arginine. On the basis of their finding concerning arginine and their previous results indicating the value of simple,

<sup>20</sup> Jour. Biol. Chem., 1915 (20), 445; Jour. Bact., 1916 (1), 15.

<sup>21</sup> Jour. Exp. Med., 1917 (25), 93.

<sup>22</sup> Jour. Lab. Clin. Med., 1920 (6), 96.

<sup>23</sup> Compt. Rend. Soc. Biol., 1913 (74), 272.

straight-chain amino acids, such as glycine,<sup>24</sup> they propose the following medium for routine cultivation of the organism, claiming that the bacilli are not only morphologically like those cultivated on glycerol-peptone bouillon, but are also able to maintain their virulence through prolonged cultivation upon it, and produce in it a potent tuberculin: Water, 1000 cc.; sodium chloride, 5.0 gram; potassium acid phosphate, 5.0 gram; magnesium citrate, 2.4 gram; glucose, 4.0 gram; glycine, 4.0 gram; arginine, 1.2 gram; glycerol, 40.0 gram; reaction equal to N/25000 NaOH.

In another report<sup>25</sup> the same authors concluded that two sources of nitrogen are necessary, (1) diamino acids or extractives and (2) mono-amino acids. Creatinine, creatine, carnine, carnosine and guanidine were found to be valuable growth promoters. Their place, however, could be taken by arginine. The purine bases were considered of no importance to growth, as the addition of guanine, xanthine, adenine, hypoxanthine or uric acid, added nothing to the growth in flasks containing only mono-amino acids. Allantoin, alloxan, barbituric acid and cyanuric acid also failed to increase growth.

Meyer and Schaeffer<sup>26</sup> in a later report have attempted to explain the interchangeability of the muscle extractives and the diamino acids. The explanation probably lies in the mutual presence of either the imidazol nucleus, which is present in histidine and carnosine, or the guanidine nucleus, which is present in arginine and extractives of the creatine type. The exact rôle of these nuclei in the nitrogen economy of the organism is not known, but the authors refer to the indications obtained by Hopkins and Ackroyd<sup>27</sup> that those nuclei are important in purine metabolism. Meyer and Schaeffer think they may be concerned in the synthesis of purines, but why they are so much superior in this respect to the purines themselves is not clear.

Long,<sup>28</sup> considering as established facts the usefulness of amino acids and their deaminization to some extent in the course of

<sup>24</sup> Jour. physiol. et path., 1913 (15), 797.

<sup>25</sup> Compt. Rend. Acad. Sci., 1912 (154), 537.

<sup>26</sup> Compt. Rend. Soc. Biol., 1919 (82), 113.

<sup>27</sup> Biochem. Jour., 1916 (10), 551.

<sup>28</sup> Trans. of the 16th Annual Meeting of Natl. Tuberc. Assoc., 1920.  
Amer. Review Tuberc., 1922 (5), 857.

metabolism, has attempted to learn the method by which ammonia is liberated from them, believing that the liberation of the latter on the one hand and an organic acid on the other, may account for the well known reaction changes of the culture medium. Amino acids may be deaminized by oxidation, reduction or hydrolysis, with the production respectively of the ammonium salt of a ketone, a normal fatty or an oxy acid. They also may be decarboxylated with the production of an amine. Assuming that if any of these substances are formed from an amino acid which serves as sole source of nitrogen, they in turn should be likewise capable of serving as the only source of nitrogen, he grew the bacillus first on an alanine glycerol medium, and then replaced the alanine in a series of experiments by ammonium pyruvate, propionate and lactate, that is, the ammonium salt of the ketone, fatty and oxy acids derivable from alanine. The ethyl amine medium did not support growth and was ruled out as a product of normal metabolism. In this connection it is interesting to note that Hanke and Koessler<sup>29</sup> did not obtain decarboxylation of histidine with the tubercle bacillus, finding that it was broken down by a different route, imidazol propionic, pyruvic or lactic acids. Long also failed to obtain growth when ammonium propionate was substituted for alanine. Growth occurred when ammonium lactate or pyruvate was used. Just why ammonium propionate repeatedly failed to support growth, even in the presence of glycerol as a source of carbon, when other ammonium salts were effective, is not clear. Ammonium propionate is suitable as a complete source of both nitrogen and carbon for some organisms. But the propionate ion seemed to inhibit the growth of the tubercle bacillus; so reduction of alanine, which would have produced propionic acid, was considered ruled out as a method in the normal metabolism of this amino acid. This leaves the lactate and pyruvate as the possible intermediates, and attempts are in progress to isolate them in the medium of culture on alanine.

In the course of the same investigation Long tested growth on a number of other amino acids and nitrogenous compounds. Leucine was a suitable sole source of nitrogen, as was histidine. The amino acids containing the benzene ring, tyrosine, phenyl-

<sup>29</sup> Jour. Biol. Chem., 1922 (50), 131.

alanine and tryptophane, were not. They possess a free amino group, but in all probability the compound left on its abstraction is inhibitory to growth. Propionamide was as well used as alanine. Creatinine, which was used as a compound containing imino but not amino nitrogen, did not support growth—a different result from that obtained by Armand-Delille and his collaborators. Urea, as has been noted by several other observers, was likewise unutilizable.

## CARBON METABOLISM

As was pointed out above, the tubercle bacillus is distinguished from other organisms by its marked glycerophilism. This property, discovered by Nocard and Roux,<sup>30</sup> is shared apparently by all three types of true tubercle bacilli, although Kendall<sup>31</sup> has questioned the extent of the utilization of glycerol in the metabolism of bacilli of the bovine type. It is worthy of note that the tubercle bacilli of cold-blooded animals, such as frog, fish and turtle bacilli, are not especially glycerophilic. Distinctions between members of the acid-fast group will be considered below in the section of this chapter on metabolic products, and in Chapter III on the other acid-fast organisms. The medium on which the tubercle bacillus was first isolated, coagulated blood serum, was low in, but not free from, glycerol. The fat content of serum varies from a low fraction of 1 per cent in starvation to 30 per cent in the extreme lipemia of some cases of diabetes. It is always increased by the ingestion of a meal rich in fat.

Since Nocard's introduction of its use, glycerol has been considered indispensable and is never left out in the routine cultivation of tubercle bacilli. Such a striking requirement naturally could not fail to attract the attention of chemical bacteriologists who had any experience with *B. tuberculosis*, and not a few investigations have been concerned with its explanation. In spite of this, the question of the exact rôle of glycerol remains today unsolved.

The pioneer investigator of the chemistry of the tubercle bacillus, Hammerschlag,<sup>32</sup> was impressed by the glycerol require-

<sup>30</sup> Ann. Inst. Pasteur, 1888 (1), 19.

<sup>31</sup> Jour. Infect. Dis. 1920 (26), 77.

<sup>32</sup> Zentr. klin. Med., 1891 (12), 9.

ment of the organism. His analyses showed that in the course of growth a notable utilization of this substance took place, while sugars were hardly touched. The same result was obtained by Bouveault.<sup>15</sup>

Proskauer and Beck<sup>17</sup> attempted to replace glycerol in synthetic media of known chemical composition with other sources of carbon. Investigation showed that other polyatomic alcohols, glycol, erythritol, dulcitol and mannitol, could not be substituted for glycerol. Nor could a variety of sugars tried, glucose, levulose, mannose, maltose, lactose, sucrose or raffinose. Nevertheless, Proskauer and Beck were of the opinion that a number of these compounds, while unable to act like glycerol, as the sole source of carbon, were distinct growth increasers. This was especially true of glucose, mannose and mannitol. Since their report several investigators have used glycerol-mannitol media for the production of bacilli in quantity from "synthetic" media. Proskauer and Beck noted that neutral salts of certain polybasic acids also, especially citric and tartaric acids, were favorable to growth.

Tiffeneau and Marie<sup>18</sup> were unable to verify Proskauer and Beck's impression concerning the value of mannitol, finding it quantitatively recoverable from old cultures. They recommended a glycerol content in the medium of 2.5 per cent, reporting a utilization of this substance to the extent of 1.5 grams per 100 cc. in the course of the usual growth. Baudran,<sup>33</sup> commenting on this simultaneous disappearance of glycerol and phosphoric acid from the medium, suggested that they combined to make glycerophosphoric acid, useful for the synthesis of phosphatids.

Kendall, Day and Walker,<sup>34</sup> in studying the metabolism of tubercle bacilli in a modified Uschinsky medium, used an organism able to multiply with either glycerol or mannitol as a source of carbon. It is worthy of note, however, that this organism was characterized as a "saprophytic" tubercle bacillus, having lost its virulence in the course of years of culture on artificial media. A finding of great importance by these authors was the fact that sugars and

<sup>33</sup> Compt. Rend. Acad. Sci., 1910 (150), 1200.

<sup>34</sup> Jour. Infect. Dis., 1914 (15), 428.

glycerol in the media exerted no protein-sparing action. This indicated that proteins were valuable for structural purposes and were not being attacked to an appreciable extent to supply necessary energy.

Armand-Delille, Mayer, Schaeffer and Terroine<sup>24</sup> repeated much of Proskauer and Beck's work on attempted substitution for glycerol and made some new observations. They were unable to secure growth by replacement of the glycerol with ethyl alcohol, glycol, erythritol, dulcitol, mannitol, glucose, maltose, sucrose or soluble starch, nor were glycerose or tartronic acid suitable. One is naturally interested in this last finding because of the close chemical relationship of these two compounds to glycerol. The authors give their results without comment and too briefly to permit conclusions to be drawn on this important finding.

Long<sup>9</sup> was unable to grow the tubercle bacillus well in the absence of glycerol. He found, however, as had been pointed out by Proskauer and Beck, that in culture in the presence of glycerol the addition of the ammonium salts of dibasic acids was distinctly favorable. Ammonium oxalate, malonate and succinate were found to promote growth, as did ammonium malate and tartrate. In a later report,<sup>28</sup> concerned more with cultural distinctions among a variety of acid-fast organisms, he confirmed the general impression that the carbonaceous residue left after the abstraction of nitrogen from amino acids, no matter by what route of degradation that took place, was unsuitable for growth as a sole source of carbon, and in some cases was distinctly unfavorable to the growth when a suitable source of carbon, glycerol, was present. For instance, while alanine was a sufficient source of nitrogen it was an insufficient source of carbon. Its possible modifications, ammonium lactate, pyruvate and propionate, and the ethyl amine, were likewise unsuitable as sole organic constituents, although supporting growth in that capacity for a number of other acid-fast organisms. However, when glycerol was added to alanine or ammonium lactate and pyruvate solutions, growth occurred. The ammonium propionate-glycerol combination was still unsuitable. He also made some observations on the inhibitory influence of aromatic rings, when present in high proportion, finding that while M/10 alanine in the presence of glycerol sup-

ported growth, M/10 phenyl alanine did not, and that when a part of the alanine was substituted with phenyl alanine in such amount that nitrogen was present in the same concentration as in the two preceding experiments, the growth was much less. It was believed that the failure of growth represented inhibitory influence of the ring compound, rather than an inability to abstract nitrogen from the phenyl alanine, the configuration of which, as far as the nitrogenous part of the molecule is concerned, is exactly similar to that of alanine.

The observation of Frouin<sup>35</sup> that growth, though distinctly lessened in amount, will still occur if the glycerol is replaced by glucose, levulose, lactose or maltose in 15 per cent concentration, brings up an important point. The product of the smaller growth which did take place under these unfavorable conditions was of different chemical composition than that obtained when glycerol was present, the lipin content being cut from 30 to 40 per cent down to 7 to 14 per cent. Glycerol is thus a lipin builder, and as the lipin of the tubercle bacillus is largely wax, it is probably a wax builder, not merely a ready-made constituent of glycerides. It is unfortunate that no qualitative comparison of the lipin of Frouin's bacilli from glycerol-containing and glycerol-free media was made.

Long and Campbell<sup>36</sup> found that the bacilli of the acid-fast group, grown upon glycerol-peptone broth or agar, produced wax roughly in proportion to their glycerophilism. They look upon glycerol as a wax-, not a fat-progenitor. The lipin of grass bacilli, which grow readily without glycerol, was found to contain only 5 to 10 per cent wax (difficultly saponifiable lipid) while that of true tubercle bacilli, which will not grow well without glycerol, was 60 to 80 per cent wax.

In considering the rôle of glycerol in the nutrition of the tubercle bacillus its physical effect should not be lost sight of. Nocard added it to media originally as a hydrophilic agent. Masucci has compared the osmotic pressure of plain and 5 per cent glycerol-peptone broths, finding them respectively 7.2 to 10.8 and 31 atmospheres.

<sup>35</sup> Compt. Rend. Soc. Biol., 1921 (84), 606.

<sup>36</sup> Amer. Review Tuberk., 1922 (6), 636.

Very little work has been done on the utilization of sugars. In the first place, growth is not nearly so good with sugar as with glycerol as a source of carbon, and when the two are combined the presence of the decomposition products of glycerol may render the estimation of sugar uncertain. Hammerschlag<sup>32</sup> and Bouveault<sup>33</sup> reported that no great utilization took place. Theobald Smith,<sup>37</sup> however, found by the fermentation test with *B. coli* that growth of tubercle bacilli for two months in dextrose broth removed in one case 50 per cent and in another nearly all of the sugar. Gamble and Herrick,<sup>38</sup> using the Folin blood sugar colorimetric method on dextrose broth in which tubercle bacilli had been cultivated three months, found a utilization of dextrose by all of five strains of bacillus, including human, bovine and avian types, of from 12-80 per cent of the original content (from 130 to 190 mgm.). Sugar utilization was roughly parallel to the amount of growth.

## MINERAL NUTRITION

Inorganic materials are indispensable to protoplasm. They play an important rôle in osmotic pressure relations, and, furthermore, the protein is to a certain extent in the salt form, weakly basic in some cases, weakly acid in others. Chapter I may be consulted for a list of the inorganic materials which do occur in the protoplasm of the tubercle bacillus, while a short consideration will be accorded here to those which exact investigation has shown must be present in the medium for growth to occur.

In the first place, phosphates are indispensable. Growth never occurs in the absence of phosphorus. It is, of course, essential in the formation of the nucleic acid of nucleoprotein and the glycerophosphoric acid of the phosphatids, both of them unquestionably vital to the cell.

More or less discussion has centered around the necessity of other inorganic ions. According to Löwenstein,<sup>39</sup> potassium, sodium, chlorine and sulphur are not essential to growth, this investigator reporting growth on a medium containing only am-

<sup>37</sup> Jour. Med. Res., 1910 (18), 185.

<sup>38</sup> Amer. Review Tuberc., 1922 (6), 44.

<sup>39</sup> Cent. f. Bakt., 1913 (68), 591.

monium phosphate, glycerol and water. Such results must be scrutinized carefully in view of the fact that it is difficult to maintain a culture medium free from these elements, as all of them may be constituents of glass. For exact research on this problem pure quartz or platinum containers should be used.

Sauton<sup>40</sup> grew the bacillus on a synthetic medium containing potassium phosphate, magnesium sulphate and ferric citrate as sources of inorganic material. He considered potassium essential, as it could not be replaced by other members of its chemical group, lithium, caesium or rubidium.

Lockemann,<sup>41</sup> whose work should be consulted for a review of the subject, found that sodium, calcium, chlorine and sulphur were non-essential to growth, but that potassium and magnesium were indispensable. According to Lockemann the *sine qua non* of growth of the tubercle bacillus is glycerol, a nitrogenous substance, phosphoric acid, potassium and magnesium.

Baudran<sup>42</sup> has tested the limiting concentrations of certain metallic ions and found that the tubercle bacillus will not tolerate iron and manganese in higher concentration than approximately 0.3 per cent. Frouin<sup>43</sup> found that the introduction of the sulfates of cerium, lanthanum, neodymium, praseodymium, and samarium in 1:40,000 concentration reduced growth 35 to 40 per cent and reduced the fat and wax content of the bacilli.

#### GAS METABOLISM

Only a few experiments dealing with the respiratory gas exchange of the tubercle bacillus are on record. Oxygen is of course absorbed and carbon dioxide given off, but the respiratory quotient on different media has apparently not been determined. As is well known, the tubercle bacillus is an almost strict aerobe. Oxygen pressures much over normal inhibit growth, however. Moore and Williams<sup>43</sup> have shown that when the percentage of

<sup>40</sup> Orig. Com. 8th Intern. Congr. Appl. Chem., 1912 (19), 267.

<sup>41</sup> Cent. f. Bakt., 1919 (83), 420.

<sup>42</sup> Compt. Rend. Acad. Sci., 1920 (170), 1471.

<sup>43</sup> Biochem. Jour., 1909 (4), 177, and 1910 (5), 181.

oxygen exceeds 70 per cent of an atmosphere, growth proceeds poorly. Adams<sup>44</sup> has confirmed this.

An important observation is that carbon dioxide, detrimental as it is to growth when in moderate to high concentration, is not only desirable but necessary before growth may occur. Tiffeneau and Marie<sup>48</sup> found it advantageous to add a little sodium carbonate to the medium, an observation which was later confirmed by Frouin.<sup>45</sup> Wherry and Ervin<sup>46</sup> determined the effect of varying concentrations of carbon dioxide upon the growth of two strains of the tubercle bacillus. They found that oxygen diminished and carbon dioxide accumulated during growth in a closed system. No growth occurred in the absence of carbon dioxide or even at the room partial pressure of 7.7 mm. It began with a pressure of 17 mm. and was inhibited in turn when the pressure passed 47 mm. The authors explain the well known lag between the time of inoculation and the first appearance of growth as the time required for carbon dioxide, produced in the course of the metabolism of the bacillus, to accumulate up to the desired concentration.

Corper, Gauss and Rensch<sup>47</sup> have correlated carbon dioxide inhibition of bacillary growth with animal and tissue resistance to the disease. Their paper should be consulted for a discussion of previous conceptions on this subject. These authors confirmed the observation of Wherry and Ervin on the absolute necessity for some carbon dioxide, noted that growth occurred with the normal air pressure of carbon dioxide of 0.04 per cent, found beginning inhibition of growth at 3 per cent and complete inhibition at 6 per cent and above. They found that the growth of tubercle bacilli in a closed system was self limited by the accumulation of carbon dioxide from their metabolism, which might reach a concentration of 5.5 per cent. They point out a significant feature in this connection, namely, that this concentration occurs in the animal body, and they were able to demonstrate an inhibitory effect upon growth by burying inoculated tubes in the

<sup>44</sup> Biochem. Jour., 1912 (6), 297.

<sup>45</sup> Compt. Rend. Soc. Biol., 1913 (74), 1184.

<sup>46</sup> Jour. Infect. Dis., 1918 (22), 194.

<sup>47</sup> Amer. Review Tuberc., 1921 (5), 562.

subcutaneous tissues of dogs, thus subjecting the bacilli in the tubes to the carbon dioxide content of the dogs' tissues. Tubes similarly buried, but connected with atmospheric air, showed excellent growth.

#### PRODUCTS EXCRETED—THE REACTION CURVE

Theobald Smith, who in 1898 distinguished the etiological agent of cattle tuberculosis as a separate type of tubercle bacillus, was the first to make more than superficial comment on the reaction changes media went through in the course of tubercle bacillus cultivation. Furthermore, although admitting that many exceptions occurred, he put forward the thesis that human and bovine types of bacillus could be distinguished on the basis of their reaction curves. He summarized the work of previous years, commencing in 1905, in an article published in 1910.<sup>37</sup> His method was to grow the bacilli on the surface of glycerol bouillon, using 100 cc. amounts in flasks of uniform size and titrating the contents from time to time with phenol phthalein as indicator. With both types of organism there was an initial progression toward alkalinity, which ceased after some weeks and gradually gave way to a developing acidity. With the bovine type, in typical cases, this acidity did not tend to become greater than the original acidity of the medium, while cultures of the human type became considerably more acid than at the start. Figures 1 and 2 record some of Smith's results on typical cases.

Smith explained the reaction curve as follows. At first the bacilli produce more alkali than acid, the latter being neutralized and used up. When the fluid becomes covered with a membrane, or the still incomplete membrane becomes quite thick, as is usually the case with human type bacilli, the tendency to produce acid becomes greater because of the partly anaerobic conditions. It was suggested as an explanation for the difference in behavior of the two types that the bovine variety utilizes the glycerol without splitting it into acids whereas the human type breaks it up into acid products.

Since Smith's first report the value of the method as a means of distinguishing between human and bovine types has been seriously questioned, the exceptions to typical behavior being far in excess

of those required to prove the rule. A. S. Griffith<sup>48</sup> of the British Royal Commission on Tuberculosis found that the final reaction of all strains could be so grouped as "to form an unbroken series in which there is nowhere a gap that would justify the conclusion that two essentially different kinds of organisms are dealt with." He found that in litmus milk rapidly growing human type strains produced an initial alkalinity, after which the milk turned acid and coagulated. With less vigorous growth acidity developed without coagulation. Rapidly growing bovine type bacilli turned milk acid but did not coagulate it. Poorly growing strains left it alkaline. In other words, acid production was to be correlated with luxuriance of growth rather than type of organism, with recognition of the fact that the bovine organism was a less vigorous grower.

Park and Krumwiede,<sup>49</sup> in the course of an elaborate investigation which should be consulted for a thorough comparison of human and bovine types, obtained results agreeing essentially with those of Smith. They agreed with Lewis and Griffiths<sup>50</sup> that no marked biological difference is concerned, and commented upon the limitations of the method imposed by the time consumed in making the test. Marie Grund,<sup>51</sup> who extended their work, says, "The glycerol reaction curve is undoubtedly a valuable corroborative evidence of a division of tubercle bacilli into two types. Its value is lessened, however, by the number of irregular and atypical reactions encountered and it is handicapped by the length of time required to carry it out."

Wankel,<sup>52</sup> who reviews the literature on the subject briefly, states that a majority of observers have been unable to confirm Smith's claims concerning the test as a means of distinguishing types of organism. In his own experience he found that of 25 cultures of tubercle bacilli of known human type, 11 gave the curve described by Smith as characteristic for the human type and 6

<sup>48</sup> Royal Commission on Tuberculosis (Second Interim Report), 1907 (3), 55.

<sup>49</sup> Jour. Med. Res., 1910 (23), 205.

<sup>50</sup> Jour. Exper. Med., 1910 (12), 82.

<sup>51</sup> Jour. Med. Res., 1911 (25), 335.

<sup>52</sup> Deut. med. Woch., 1913 (39), II, 2461.

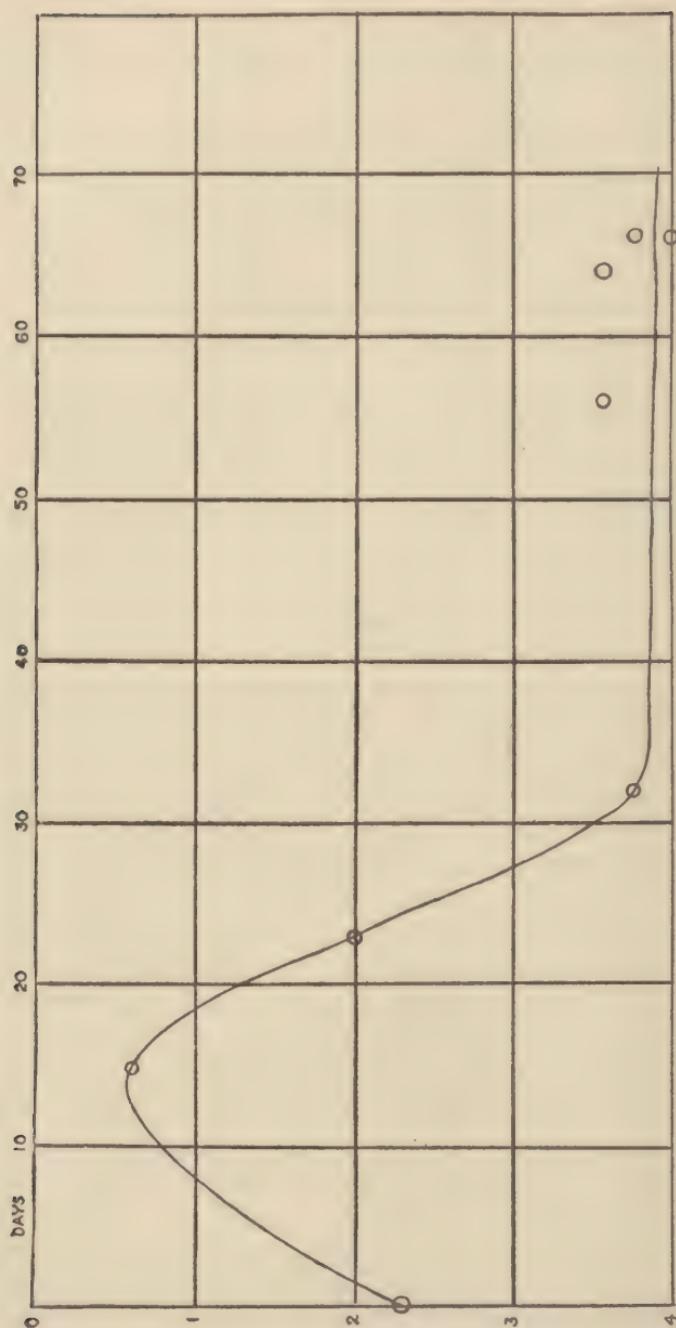


FIG. 1

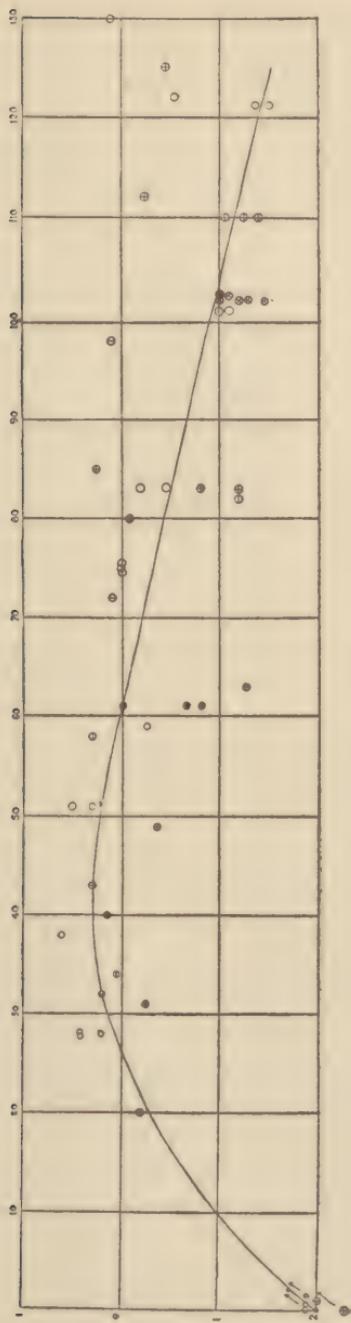


FIG. 2

gave the bovine type curve, while the rest were doubtful. Of 20 bovine strains, 15 gave the bovine type curve, 4 gave typical human type curves, while 1 gave a curve midway between the two. Thus a majority of cases behaved in the manner described by Smith as typical, but the incidence of exception was high, as it should be pointed out, Smith himself admitted.

More recently Frothingham<sup>53</sup> has followed the reaction curve of tubercle bacillus cultures by more modern methods than the titration used by Smith, determining the hydrogen-ion concentration at various stages of growth by means of the potentiometer. He found that curves obtained by this method corresponded to the Smith curves and showed the same discrepancies as respects differentiation of type. Some strains gave different curves on repeated tests. For H<sub>37</sub>, a characteristically human type bacillus, he obtained a curve the acid and alkaline limits of which may be calculated from his figures as pH 8.15 and 5.52. A little later Frothingham<sup>54</sup> studied the growth of tubercle bacilli on media to which small amounts of indicator had been added. Using brom-cresol-purple he found that glycerol broth culture media of human type bacilli tended to become acid while those of the bovine type remained alkaline. He concluded that this indicator might be useful in determining H-ion concentrations of culture media of tubercle bacilli and in distinguishing human and bovine types.

Kendall, Day and Walker,<sup>55</sup> comparing the metabolism of virulent human type bacilli and bovine type bacilli, obtained, for the former, results somewhat different from their previous results on avirulent cultures. The development of an acid reaction by human type bacilli together with minimal deaminization, as shown by a decrease in the ammonia content of the medium during the period of growth suggested that the glycerol is utilized largely for the energy requirements of the bacilli, yielding acid products in its breakdown, but shielding the protein constituents of the medium. In the metabolism of bovine type bacilli, on the other hand, protein shielding was not in evidence. The development and persistence of an alkaline reaction, with unmistakable evidence

<sup>53</sup> Jour. Med. Res., 1917 (37), 269.

<sup>54</sup> Jour. Med. Res., 1918 (39), 153.

<sup>55</sup> Jour. Infect. Dis., 1920 (26), 45 and 77.

of cleavage of protein to amino acids and deaminization of the latter, indicated to the authors that bovine type bacilli do not ferment glycerol. Their results thus tended to bear out Smith's thesis and offered an explanation of the difference in the reaction curve of the two organisms.

Long and Major<sup>56</sup> devised a method of following reaction changes, which is simple and time and space saving, as compared with the laborious Smith titration method and records the reaction in terms of hydrogen-ion concentration in a manner similar to that suggested by Frothingham. The method is as follows:

Four test tubes, 1 inch wide, each containing 10 cc. of glycerol-peptone broth or any other liquid medium, neutral in reaction (pH 7.0), plus 0.001 per cent phenol-sulphonephthalein, are inoculated with plaques of tubercle bacilli, approximately 0.75 cm. in diameter, sealed with tin foil, and incubated at 37.5°C. Once a week the tubes are taken out and the color compared with standards of known reaction containing the same concentration of indicator. The Dernby and Avery<sup>57</sup> comparator is used with a tube of broth back of the standard when determining broth reactions, to compensate for the color of the medium. Apparently at this concentration the indicator does not inhibit the growth of these organisms. Care must of course be taken that the plaque remains floating. The reactions of the four tubes are averaged in plotting the curve. By the use of this method curves were obtained in seven weeks' time for two strains of organism isolated from human and bovine lesions, respectively almost avirulent and highly virulent for rabbits, which approximated very closely the typical curves of Theobald Smith. However, a recently isolated human type strain, H<sup>104</sup>, gave a characteristic bovine type curve at the same time. The curves are given in figures 3, 4, and 5.

These investigators<sup>58</sup> later modified their method so that organisms which will not float may be used. The method is essentially the same, except that 2 per cent agar is added to the medium and a tube containing agar but no indicator is placed back of the

<sup>56</sup> Amer. Review Tuberc., 1921 (5), 715.

<sup>57</sup> Jour. Exp. Med., 1918 (28), 345.

<sup>58</sup> Long, Campbell and Smith (Agatha Major), Trans. of the 18th Annual Meeting of the Natl. Tuberc. Assoc., 1922.

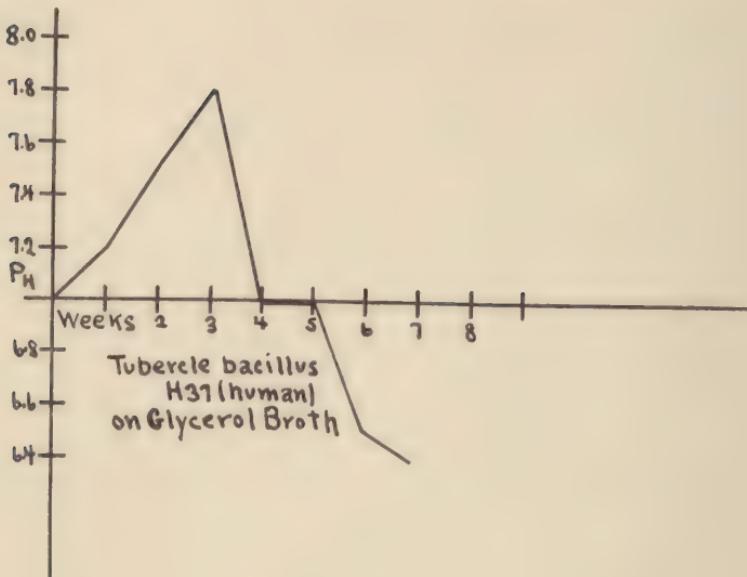


FIG. 3

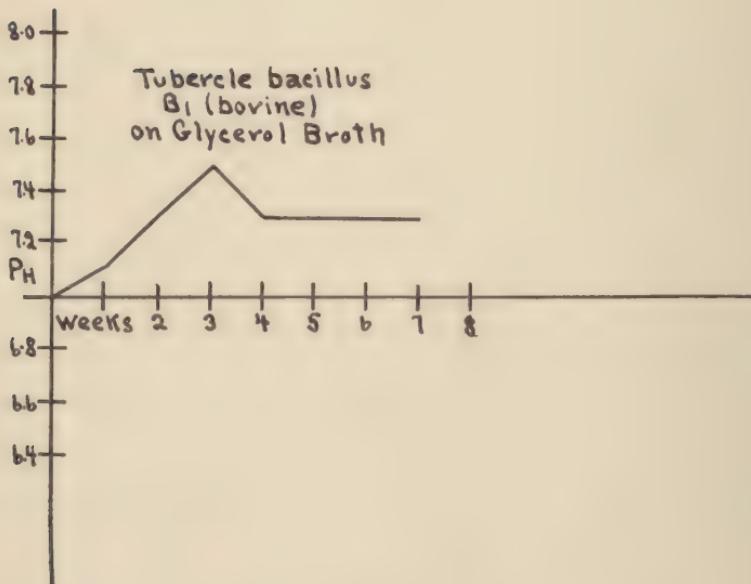


FIG. 4

standards to compensate for the turbidity of the agar. They found that diffusion through the agar was sufficiently rapid to render the method fully as useful as the preceding, for all types of bacillus, floating or not. They recorded that all bacilli of the acid-fast group produced alkalinity from amino acids, acid amides, and ammonium salts, but that this might be masked by acid production in the presence of glycerol.

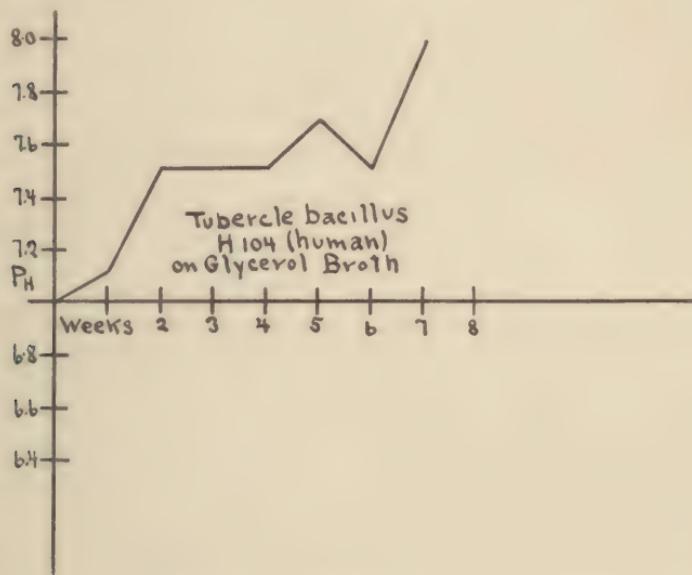


FIG. 5

Long<sup>9</sup> has on another occasion noted a terminal acidity in cultures of the same bovine type bacillus as referred to in figure 2, the degree of which depended on the original reaction. In this case in comparing the growth of human and bovine type strains on glycerol broth he obtained an increase in acidity in both types which was distinctly greater with the human type and definitely correlated with greater luxuriance of growth. Figures 6 and 7 record the growth of the two strains, run in triplicate, and the initial and final reaction. They show that growth of the tubercle bacillus may proceed readily between an initial H-ion concentration of pH 6.4 and one of 7.8, although some inhibition of growth of the bovine type is seen at pH 6.4.

Whether the reaction curve is a practical means or not for distinguishing types of organisms, it is of great theoretical interest for the light it throws upon the metabolism of the organism.



FIG. 6

Long<sup>59</sup> has referred the changes, not to the condition of the oxygen supply, but to cleavage of the amino acids of the peptone of the medium into ammonia and an organic acid, the rates of absorption for which may vary in such a way as to leave a preponderance of alkali or acid in the medium. In addition there is acid production from glycerol. He has drawn attention also to the fact that in the absorption of phosphoric acid for structural purposes from the neutral phosphates of the medium, a certain amount of free base is left behind, rendering the medium more alkaline. That this probably plays only a small rôle in reaction changes was

<sup>59</sup> Amer. Review Tuberc., 1921 (5), 705.

shown by his investigation with Campbell<sup>58</sup> of the rates of withdrawal of nitrogen and phosphorus from an ammonium phosphate glycerol medium by the dung bacillus (see table).



FIG. 7

*Relations of nitrogen and phosphorus utilization to reaction change in growth of dung bacillus*

Medium contains glycerol and ammonium phosphate (mixture of  $(\text{NH}_4)_2\text{HPO}_4$  and  $\text{NH}_4\text{H}_2\text{PO}_4$ )

| CULTURE NUMBER     | ACIDITY<br>$\frac{\text{N}}{10} \text{ NaOH}$ | P<br>CON-<br>TENT | P<br>LOSS | P LOSS<br>$\frac{\text{N}}{10} \text{ H}_3\text{PO}_4$ | NH <sub>3</sub><br>CON-<br>TENT | NH <sub>3</sub><br>LOSS | NH <sub>3</sub> LOSS<br>$\frac{\text{N}}{10} \text{ NH}_3\text{OH}$ |
|--------------------|---|-------------------|-----------|--|---------------------------------|-------------------------|---|
|                    | cc.   | mgm.              | mgm.      | cc.  | mgm.                            | mgm.                    | cc.   |
| Control.....       | 0   | 84.6              | 0         | 0  | 58.4                            | 0                       | 0   |
| 1 (one crop).....  | 18.5  | 80.0              | 4.6       | 1.5  | 43.5                            | 14.9                    | 8.7   |
| 2 (one crop).....  | 18.5  | 80.3              | 4.3       | 1.4  | 44.2                            | 14.2                    | 8.3   |
| 3 (three crops)... | 40.0  | 76.4              | 8.2       | 2.7  | 19.0                            | 39.4                    | 23.1  |

Phosphoric acid was withdrawn but only one-sixth to one-eighth as fast as ammonia. A notable acidity developed, about one-half of which was the result of abstraction of the base from ammonium phosphate, the other half being a product of the oxidation of the glycerol.

#### PRODUCTS EXCRETED—TUBERCULIN

Among the earlier investigations on tuberculin a number were concerned with the isolation of a specific poison, and numerous attempts were made to extract from the bacillus itself or from the medium of its growth a toxic alkaloid, the presence of which might account for the symptoms of tuberculosis. The bulk of the earlier analyses deal with tuberculin prepared from glycerol-peptone broth, which is sufficiently complicated in its original chemical composition to render the identification of minute amounts of poisonous substances in it extremely difficult. Although not a few claims are on record that poisonous substances have been extracted from raw tuberculin, it may be stated with certainty that no substance of known chemical composition has ever been obtained from the medium of growth of the tubercle bacillus which will elicit any of the symptoms of tuberculosis.

In the first place the active principle of tuberculin is not a toxin or even a poison in the usual sense of those words. It may be injected in large amounts into healthy animals without causing any intoxication. A cubic centimeter of Old Tuberculin has been given to an infant which had never had any contact with tuberculosis, without causing a reaction. The author has injected 15 cc. of a filtered four weeks' culture of a virulent bacillus into the peritoneal cavity of a normal guinea pig without causing any more ill effect than a transient irritation due to the glycerol content. On the other hand a millionth of a gram is enough to cause a manifest skin reaction in an individual suffering from tuberculosis. Obviously the toxicity of the substance depends upon the state of sensitization of the subject to whom it is given.

The tuberculin reaction is a specific response given only by an individual who has been at some time infected with tuberculosis, or with another acid-fast microorganism (see below). Such a subject, while totally unresponsive to ordinary glycerol-peptone

broth, will react to small amounts of the same medium after tubercle bacilli have been grown in it, with local hyperemia and exudation, and, if the dose is large enough, with a focal inflammation at the site of his disease and increased constitutional symptoms. The fact that a large percentage of human beings react to it in some degree merely indicates that the same percentage has been at some time infected with tuberculosis. In experimental animals, such as guinea pigs, which do not ordinarily acquire spontaneous infection, a positive reaction is diagnostic of present tuberculosis.

If, however, sensitization to tuberculin is conferred practically only by infection with tuberculosis, it is not true that only tuberculin will elicit the reaction. The same sort of response may be called forth by other protein substances, best, apparently, by certain modifications of albumoses from various substances. M. Matthes,<sup>60</sup> and Krehl and C. Matthes<sup>61</sup> found that the deutero-albumose from egg albumin, muscle and casein caused on subcutaneous injection into tuberculous guinea pigs the clinical and autopsy complex of a true tuberculin reaction, and obtained a typical response on inoculation of this material in a man with lupus. They found, however, that the amounts necessary to elicit the reaction were large as compared with the required amounts of true tuberculin. Less striking but similar results were obtained by Kircheim and Tucek,<sup>62</sup> who review the literature on this subject. Other protein products than deutero-albumose have been known to cause the reaction in tuberculous subjects, always only with larger amounts than the amount of tuberculin required to produce reactions. Apparently these preparations contain something, not identical with tuberculin, but *like* it, and in large enough amount capable of firing the train of the subjects' sensitivity.

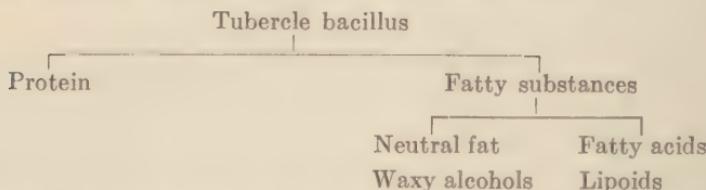
The term "tuberculin," as used in the foregoing paragraphs, is meant to indicate the broth, concentrated or not, in which tubercle bacilli have been cultivated over some weeks, including such well known forms as Old Tuberculin of Koch and B. F.

<sup>60</sup> Deut. Arch. klin. Med., 1894 (54), 39.

<sup>61</sup> Arch. exp. Path. Pharm., 1895 (36), 437.

<sup>62</sup> Arch. exp. Path. Pharm., 1914 (77), 387.

(bouillon filtrate). Before proceeding farther it is well to state that any solution in which the tubercle bacillus has proliferated with moderate luxuriance, and any extract or fraction of the bacillus containing protein, is capable of causing a "tuberculin reaction" in an appropriate subject. That the fatty substances, too, may play a rôle in the immunity reactions of tuberculosis is claimed by Much and his collaborators. According to Much<sup>63</sup> we must think of antibodies to several fractions of the tubercle bacillus, which he and Leschke<sup>64</sup> picture as follows:



Much, Deyke, Leschke, Mueller and others believe that specific immunity may be developed against each of these fractions as antigens. The antigens, which this group of investigators term "partigens," are separated from the bacillus by preliminary treatment, lasting several weeks, of finely ground bacilli in dilute organic acid, as 1 per cent laetic acid. Part of the protein goes into solution; part remains undissolved. The two fatty fractions are separated out respectively by alcohol and ether extraction. For a detailed description of methods of preparation the reader is referred to Leschke's article.<sup>65</sup> The whole subject of "partigens" and their application in diagnosis and therapy can be considered in detail in Much's book.<sup>66</sup>

In this relation we must consider the fact that Findeisen<sup>67</sup> has found that the fatty materials extracted from tubercle bacilli with chloroform, ether or xylene do not produce a tuberculin reaction in the conjunctiva of tuberculous cattle, although the bodies of the extracted bacilli still produced reactions. This seems

<sup>63</sup> Beitr. Klin. Tuberk., 1911 (20), 343.

<sup>64</sup> Ibid., 1911 (20), 353.

<sup>65</sup> Beitr. Klin. Tuberk., 1911 (20), 393.

<sup>66</sup> Die Immunitätswissenschaft. C. Kabitzsch publ., Würzburg, 1914.

<sup>67</sup> Beitr. Klin. Tuberk., 1922 (51), 237.

to be good evidence that the active tuberculin is not a lipoid nor yet a lipoid-soluble constituent of the bacilli.

The majority of immunologists, who hold that antigens are as a general rule, if not exclusively, protein in nature, believe that tuberculin in its various forms plays a rôle in immune reactions through the presence in it of protein or its cleavage products.<sup>68</sup> Many different forms of tuberculin are used for diagnostic and therapeutic purposes. Some of the chief types are filtered culture media in which tubercle bacilli have grown and been boiled, extracts of dried and ground tubercle bacilli, and suspension of dried bacilli or finely ground bacilli. For a description of the various types of tuberculin used in medical practice the reader is referred to standard works on tuberculosis.

Many of the difficulties which have beset immunologists in the interpretation of the "tuberculin reaction" have been cleared away by Zinsser,<sup>69</sup> who has shown that there are two fundamentally different types of sensitization to the products of the tubercle bacillus. The one is toward the proteins in the bacillus and is anaphylactic in nature. The other, manifested by skin hypersensitivity such as is observed in the tuberculin, mallein, and abor-tin reactions, depends upon an unidentified substance in extracts of bacilli which fails to give protein tests. That animals, particularly guinea pigs, may be sensitized to the proteins of the tubercle bacillus and thrown into anaphylactic shock by reinjection after the appropriate interval, has been demonstrated several times.<sup>70</sup> The same material that sensitizes guinea pigs in this manner will also elicit a striking skin reaction in the course of some hours when injected intracutaneously or applied to the scarified skin of a tuberculous guinea pig. Zinsser has shown that the reaction is still obtainable, however, with an alkaline extract of this bacillary protein after all *demonstrable* protein has been thrown out of solution by heat and acid coagulation. The reacting material

<sup>68</sup> Selter and Tanere (Zeit. f. Tuberk., 1921 (35), 171) report that heating tuberculin up to 150° does not modify its action upon guinea pigs. They believe this fact is not compatible with the idea that tuberculin is an antigen.

<sup>69</sup> Jour. Exper. Med., 1921 (34), 495.

<sup>70</sup> See particularly Baldwin, Jour. Med. Res., 1910 (22), 189.

is biuret free. The possibility still remains that protein is present, but in a state too dilute for recognition. Furthermore, the injection of bacillary protein, which sensitizes animals so that an anaphylactic response may be obtained later, does not ordinarily sensitize to the skin test. It is thus seen that the reactions which may be obtained with the common preparations of tuberculin are of two different types. For the purposes of the rest of this chapter "tuberculin" will be understood to mean the filtered culture media in which tubercle bacilli have been grown which will elicit the well known tuberculin skin reaction.

Obviously if tuberculin is some sort of protein substance, as the experiments with the deutero-albumose preparations indicate, and as has been generally assumed on the basis of the usual concepts in immunology, preparations of tuberculin should give protein reactions. However, these are of course of no significance in the ordinary tuberculin prepared from glycerol peptone broth, which is rich in protein as originally made up.

Koch<sup>71</sup> himself was the first to attempt the isolation of the active principle of tuberculin by chemical means, at an early date establishing the fact that the substance concerned with the reaction was precipitable by five volumes of alcohol. Although designated "purified tuberculin" by Koch, the product was unquestionably largely made up of those unmodified constituents of the original medium which were insoluble in alcohol. On the other hand it was freed from glycerol and many of the so called "extractives" of the medium. It was water soluble and gave the usual protein color reactions depending on the presence of the biuret grouping and the benzene and phenol groups, and was precipitated by phosphotungstic acid, ferric acetate, ammonium sulphate and tannic acid.

Following Koch's original preparation of tuberculin, modifications of it, far too numerous even to list, were brought forth by numerous investigators. A certain amount of chemical research was done on some of these, but the value of this is small in view of the great complexity of the materials from which the material was prepared, which were usually glycerol, meat extract and some type

<sup>71</sup> Deut. med. Woch., 1891 (17), 1189.

of peptone and proteose.<sup>72</sup> Among other investigations was a series by W. Kühne,<sup>73</sup> who came to the conclusion that tuberculin contained an albuminate, an acroalbumose, a deuteroalbumose, and traces of peptone.

The uncertainty with regard to the chemical nature of tuberculin, dependent upon the complexity of the medium from which it was prepared, naturally led not a few investigators to attempt the preparation of tuberculin from very simple materials. It was soon learned that moderately potent tuberculins could be prepared from media quite devoid of anything so complex as protein, proteose or peptone, containing nitrogen in no more complicated form than amino acids and ammonium salts. The availability of such media for the growth of tubercle bacilli and production of tuberculin was first established by Kühne.<sup>16</sup> The same year, in their elaborate work on nutrition, Proskauer and Beck<sup>17</sup> confirmed this observation that active tuberculin could be made from very simple media.

It would seem that if tuberculin is of the nature of a soluble protein excreted by the bacillus into the medium of its growth, or if it is a protein autolytic product of the organism, its presence could be easily demonstrated in the filtered culture medium by the application of simple, appropriate protein color tests. Yet the most amazing discrepancy of results has followed this line of investigation. Although practically all of the investigators cited in this chapter, who grew the bacillus upon simple, non-protein media, reported the production of active tuberculins, eliciting a typical response in tuberculous guinea pigs, they disagreed upon the presence of protein in these products.

Marie and Tiffeneau<sup>74</sup> prepared a tuberculin from a medium containing potassium phosphate, magnesium citrate, mannitol, glycerol and ammonium sulphate, the active principle of which could be freed from the other constituents of the medium by

<sup>72</sup> Jolle's chapter on tuberculin in Ott's *Chemical Pathology of Tuberculosis*, and Baldwin's chapter on the chemistry of the tubercle bacillus in the English edition of Cornet's "Tuberculosis," may be consulted for a summary of the results obtained.

<sup>73</sup> *Zeit. f. Biol.*, 1892 (29), 1.

<sup>74</sup> *Compt. Rend. Soc. Biol.*, 1909 (66), 206; 1912 (72), 48.

precipitation by alcohol, resolution in water, and dialysis, and was therefore colloidal, presumably protein, in nature.

Malm<sup>75</sup> grew tubercle bacilli on a medium containing glycerol and tartaric acid as sources of carbon, and ammonium sulphate and asparagine as sources of nitrogen, obtaining excellent growth and a filtrate which was a strong tuberculin. From this filtrate a substance having many of the properties of an albumose could be precipitated by alcohol or ammonium sulphate, which was water soluble and toxic for tuberculous guinea pigs. It gave the Heller nitric acid test and the acetic acid test for albumose. It was sulphur free and could be obtained in a sulphur-free medium.

Löwenstein and Pick,<sup>76</sup> on the other hand, obtained an active tuberculin from a medium containing only asparagine, ammonium lactate, sodium phosphate, sodium chloride and glycerol, which was heat stable, dialyzable and alcohol-insoluble, giving neither the biuret nor any other protein test.

Lockemann<sup>77</sup> used a protein-free medium containing as organic material only asparagine, citric acid and glycerol. On this medium growth reached its maximum in six weeks and then gradually diminished in weight, presumably because of autolysis; at the same time the medium developed the capacity for giving protein tests. Heat and acetic acid caused a clouding; saturation with ammonium sulphate brought down a flocculent precipitate; and potassium ferrocyanide, picric acid and tannic acid all caused precipitates. The xantho-proteic and Molisch reactions were positive, but in the Adamkiewicz test only a faint yellow color was obtained. This medium was a strong tuberculin. All reactions were more intense after eight to twelve weeks than after four weeks growth.

Long<sup>5</sup> obtained a tuberculin from a simple medium containing only ammonium chloride and glycerol as sources of nitrogen and carbon. Two tuberculous guinea pigs reacted strongly to a skin test with 0.1 cc. of the four times concentrated Berkefeld filtrate, while control healthy animals did not respond at all. This

<sup>75</sup> Cent. f. Bakt., 1913 (70), 141.

<sup>76</sup> Cited in Cent. f. Bakt., 1913 (68), 591.

<sup>77</sup> Zeit. physiol. Chem., 1911 (73), 389; Deut. med. Woch., 1913 (39), 2458.

material, in spite of its activity as a tuberculin, did not give the biuret test or any other protein test except the doubtfully significant ethyl-acetate test of Marie.

Ruppel,<sup>78</sup> who made the earliest extensive investigation of the nucleoproteins of the tubercle bacillus, maintained that the specific agent in tuberculin was a nucleoprotein. He emphasized the fact that in tuberculin, as usually prepared, this substance may be more or less profoundly modified by the heat used, and thereby rendered partially dialysable. He pointed out that Tuberculin A. F. (*albumose-free*, prepared from asparagine, citric acid, glycerol and inorganic salts) upon which Koch was working at the time of his death, is non-dialysable. Heat is avoided in the preparation of this product. Behring<sup>79</sup> credited the specific properties of tuberculin to its nucleic acid, to "tuberculinic acid."

Weleminsky<sup>80</sup> reported the production of a mucinous protein substance in the medium of very old cultures of tubercle bacilli. He noted, too, that if the product of growth of cultures eight years old was filtered off and the medium replanted with fresh cultures, growth was inhibited. The inhibiting substance, however, was thermolabile, sterilization of the medium sometimes preventing its action. He believed the slimy substance, which chemical analysis showed to be a true mucin, was a metabolite of living bacteria rather than a product of bacterial disintegration.

A number of investigators have attempted to demonstrate the protein nature of the specific substance in tuberculin by treating raw tuberculin with proteolytic enzymes. Baldwin and Levene<sup>81</sup> found that prolonged digestion of tuberculin by trypsin destroyed its activity, while pepsin only weakened it. Because of its resistance to pepsin the specific substance was thought to be a nucleoprotein. Pfeiffer and his co-workers secured similar results, noting, however, a more marked effect with pepsin. Both "old tuberculin" and "albumose-free tuberculin" were rendered inactive by trypsin and greatly weakened by pepsin. They

<sup>78</sup> Deut. med. Woch., 1913 (39), 2462.

<sup>79</sup> Berl. klin. Woch., 1899 (36), 577.

<sup>80</sup> Berl. klin. Woch., 1912 (49), 1320.

<sup>81</sup> Jour. Med. Res., 1901 (6), 120.

discuss at some length<sup>82</sup> the suggestion of Löwenstein and Pick, who, because of the failure to obtain protein reactions from a tuberculin prepared from a protein-free medium, considered the active principle a polypeptid. Pfeiffer and his collaborators were unable to substantiate this, finding that pepsin weakened the product, while erepsin failed to affect it, both results being contrary to the usual occurrence with polypeptides. By appropriate chemical measures they were able to demonstrate that the pepsin preparation they were using conformed to the standards for identification of this substance, and that to it, and not to accompanying proteases, the destructive effect upon tuberculin was due. They advance the hypothesis that preparations of tuberculin not giving the biuret test, like that of Löwenstein and Pick, are simply too dilute to give the usual protein reactions, and point out that commercial albumose-free tuberculin (T. A. F. of Höchster Farbwerke), although prepared from non-protein substance, does give the protein tests.

Danielopolu<sup>83</sup> secured essentially similar results, finding that trypsin in an alkaline medium destroyed the specific principle, and that artificial gastric juice had the same effect. The action of gastric juice could not be attributed to its acid content, as the same concentration of hydrochloric acid did not affect tuberculin. He found that tuberculous individuals who were especially sensitive to tuberculin could take ten times the dose usually causing severe symptoms if the tuberculin was previously treated with trypsin. Similar results on experimental animals have been published by Franceschelli.<sup>84</sup>

#### RECAPITULATION

Summarizing the nutritional characteristics of the tubercle bacillus discussed in the preceding pages, we note a few that are distinctive other than its marked glycerophilism. Like the majority of bacteria for which symbiotic relationships are not necessary, it derives its nourishment from compounds of compara-

<sup>82</sup> Wien. klin. Woch., 1911 (24), 1115.

<sup>83</sup> Compt. Rend. Soc. Biol., 1910 (68), 185, 896.

<sup>84</sup> Riforma Medica, 1921 (37), 914.

tively low molecular weight, obtaining such substances by fragmenting larger molecules. Yet it is not an active disintegrator of the larger organic molecules, as is shown by the fact that lipases, proteases and nucleases, while definitely proved to be present, are not very active compared with those present in the bodies of other bacteria. It may be, however, that this relative inactivity is not due to a low concentration of the respective ferments, but the result, rather, of some inhibition of their action, for experiments have indicated that ferment inhibitory substances are contained in the protoplasm of tubercle bacilli. The sluggishness of enzymic activity is possibly to be correlated with the slowness of growth of this organism. It is noteworthy, too, that in the tubercle bacillus, which is not an active utilizer of carbohydrates, those enzymes concerned with the disintegration of the polysaccharides have not been demonstrated. Catalase and those ferments concerned with reduction, which are probably universally distributed in the living world, can easily be shown to be present.

A considerable amount of investigation has dealt with its nitrogen metabolism. Experiments have shown that peptone and substances of similar protein nature disappear from the medium of growth while a certain amount of ammonia makes its appearance. Inasmuch as the tubercle bacillus uses ammonia readily and directly, and is able to thrive with ammonia as its sole source of nitrogen, it is probable that it is largely by conversion into this form that the nitrogen of the protein molecule becomes available. However it is not to be doubted that a considerable part of the protein molecule is useful, if not absolutely necessary, in the undeaminized condition. It has been shown that growth is increased if arginine is added, and there is some indication that certain of the aromatic ring compounds, in low concentration, particularly tryptophane, are similarly valuable. It is reasonable to suppose in this case that their presence in the medium saves the organism the necessity of synthesizing these indispensable portions of its protoplasm. However, it should be borne in mind that the bacillus, if called upon to do so in exceedingly simple media, is perfectly capable, if given sufficient time, of synthesizing all of them. It should be added that there is no certain evidence that it does not utilize some peptone or poly-

peptides derived from it, directly, without profound cleavage into the constituent amino acids, although it seems likely that in this case, as in that of any other cell, biologic individuality of protein would be best attained by the synthesis of the unique proteins from their simplest building blocks. This is, however, purely speculation.

Many experiments have been performed in which a single, simple source of nitrogen was present. These indicate that the amino group, either of amino acids or acid amides, is readily utilized, but that the imino group is not readily attacked, at least in the absence of other more readily assimilable nitrogen on which the bacillus may make a good start. Substituted amino groups, as  $-\text{NH}(\text{CH}_3)$  seem unutilizable. Primary amines apparently are not attacked, nor do they seem to be formed in the course of growth. This is a point of importance in view of the well known physiological activity of some of these substances. The nitrogen of the purine bases, even the amino purines, does not seem abstractable; at any rate the organism does not proliferate when these compounds are supplied as its sole source of nitrogen. It may be, however, that in this case, as seems to be true when such ring compounds as tryptophane and tyrosine are used, the resultant deaminized residue is inhibitory. Experiments to prove this do not appear to be on record.

The most characteristic nutritional requirement of the tubercle bacillus is that for glycerol. However useful other carbonaceous groupings may be in combination with glycerol, growth is never luxuriant in the complete absence of this substance. The explanation of this glycerophilism is not definitely known, but there is strong indication that glycerol plays a rôle in the synthesis of the organism's wax. The wax, it is to be noted, is not a glyceride, but an ester of a monatomic alcohol of high molecular weight. There is some evidence that other alcohols than glycerol, particularly those of the hexose group, are an aid to growth. That is, they are apparently metabolized. However, glycol, erythrol, dulcitol and mannitol cannot replace glycerol for good growth; only the triatomic alcohol is effective. Growth may indeed occur with other substances as sole source of carbon, as glucose in 15 per cent concentration but it is relatively scanty and the bacilli

are different from those obtained on glycerol media, being inferior in lipin content. It should not be forgotten that glycerol plays also a physical rôle in the ordinary culture media, being hydrophilic and exerting in the concentrations used a considerable osmotic pressure.

There is nothing distinctive in the mineral requirements of the tubercle bacillus. Phosphate is necessary as in all cells, for the construction of nucleic acid and phosphatids. Potassium and magnesium are said to be indispensable and the only other inorganic ions which truly are indispensable, but it seems reasonable to suppose that an abundant supply of other ions will aid in preserving normal osmotic pressure and in making certain combinations with proteins known to occur in all cells.

The organism is almost strictly aerobic; but oxygen pressures much above normal inhibit growth. A small amount of carbon dioxide is also necessary for the initiation of development. Higher concentrations, however, beginning with 3 per cent, are distinctly inhibitory.

In the course of the metabolism of the tubercle bacillus changes occur in the reaction of the medium of its growth which have been considered by various investigators, notably Theobald Smith, as characteristic for given strains, and particularly valuable in the distinction between bacilli of the human and bovine types. Considerable exception has been taken to the claim that such changes are specific for given strains, especially in view of the fact that a number of results have indicated that reaction change, particularly in the direction of acidity, was to be correlated with luxuriance of growth more than with the type of bacillus. It seems that this question will remain unsettled until the chemical conditions for the change are better understood. In the meantime virulence tests on laboratory animals will remain the standard means of differentiating types.

The reaction change in glycerol-peptone (or amino acid) media I have explained on the following basis. Splitting of amino acids, either by oxidation or hydrolysis, into ammonia and an organic acid, followed by combustion of the acid for energy, or its synthesis into bacillary protoplasm, will, if the ammonia is not synthesized into bacterial substance at a corresponding rate,

leave the medium alkaline. On the other hand if the withdrawal of ammonia is the more rapid the medium becomes progressively more acid. Organic acids are produced by oxidation of the glycerol. The oxidation products of glycerol have not been isolated. There remains one other source for alkalinity; phosphoric acid may be withdrawn from the neutral phosphates present, for the construction of nucleic acid and phosphatids, leaving a corresponding amount of alkali behind. The net effect of this has been shown to be small. The result of all those factors in a typical case of a human type bacillus in glycerol-peptone broth is a primary increase in alkalinity of the medium, followed by a decrease, with finally a production of acid in such quantity as to render the medium appreciably acid in reaction, perhaps with a hydrogen ion concentration approximating pH 5.0. With a considerable number of bovine type strains the acid production is not so great, the primary alkalinity being followed by no more than enough development of acid to return the medium to approximately its original reaction (Theobald Smith curve). Attempts are at present in progress to reproduce such curves with simple media of accurately known chemical composition.

The substances excreted by the tubercle bacillus include more, however, than the relatively simple metabolic products of protein and glycerol just referred to. In the course of growth there passes from the bacillus into the medium a substance of unknown chemical composition toward which the tuberculous organism is specifically sensitive, "tuberculin." While other substances than tuberculin are capable in moderate concentration of eliciting a similar reaction, particularly certain varieties of proteose, it does not appear that tuberculin excites a response, in the usual concentrations employed, in any organisms except those at one time infected with the tubercle bacillus. Such a specificity would not unnaturally be attributed, on the basis of the prevalent conceptions in immunology, to the presence in tuberculin of protein unique for the tubercle bacillus. The manner in which such a protein excites a specific response has not as yet been chemically defined. The reaction has been generally assumed to be allergic in nature. One of the more prevalent explanations of allergy, with which the names of Vaughan and Friedberger and Abder-

halden are associated, assumes that a foreign protein, in this case the tubercle bacillus itself, stimulates the elaboration within the infected organism of ferments acting specifically upon the foreign protein in question. Whenever this protein is introduced in such a sensitive organism, as in the application of the tuberculin skin test, according to this explanation, it is split by the specific ferments of the organism, with the production of at least one toxic substance, which is responsible for the local reaction. Experiments are on record indicating that the protein of raw tuberculin when treated by the method of Vaughan does yield poisonous substances which on injection into a normal animal elicit a response similar to that obtained with the untreated tuberculin in a tuberculous animal.<sup>85</sup>

However it is yet to be proved to the satisfaction of everybody that tuberculin<sup>86</sup> necessarily contains a protein substance.

Zinsser has shown conclusively that extracts of tubercle bacilli from which all demonstrable protein has been removed by heat and acid coagulation, are quite capable of eliciting a skin reaction in a tuberculous animal; he has emphasized the fact that this type of reaction is to be sharply distinguished from the *anaphylactic* response which may be obtained with tuberculo-protein.

The usual preparations of tuberculin, made from glycerol-peptone broth, contain a large amount of protein which was present before the growth of the bacillus. A satisfactory demonstration of the protein nature of tuberculin requires the production of protein by the growth of the bacillus in protein-free media. As shown in the text above the bacillus will proliferate in simple, non-protein media of known composition, in which an amino acid or ammonium salt supplies all of the nitrogen, and there is no question that the filtered, bacteria-free medium in these cases is a moderately active tuberculin. While the majority of investigators have found that tuberculins thus prepared contain a substance responding positively to the usual protein tests, a number

<sup>85</sup> White, Trans. Ninth Annual Meeting Nat'l. Assn. Study and Prev. Tuberc., 1913, 315; White and Avery, Jour. Med. Res., 1912 (26), 317.

<sup>86</sup> "Tuberculin" is here used to mean the bacteria-free medium in which tubercle bacilli have been grown, which elicits a typical skin response in a tuberculous animal.

have been able to call forth typical tuberculin reactions with preparations not giving any of the protein color or precipitation reactions. It may be in these cases, as Pfeiffer assumes, that the solutions used are simply too dilute in protein to give the chemical tests, although containing sufficient to elicit the much more delicate biologic reactions. There are others who believe that tuberculin may be protein in nature to the extent that it is composed of combined amino acids, but that these are in the form of relatively simple polypeptides not sufficiently complex to give those color reactions depending on the presence of certain aromatic rings. This seems doubtful. It remains to be proved, moreover, that any such simple compounds are antigenic. The possibility still remains that the active agent of tuberculin may not be protein at all, and that the appearance of protein following the growth of tubercle bacilli in non-protein media may be a purely coincident phenomenon.

## CHAPTER III

### ACID-FASTNESS

The phenomenon of acid-fastness makes possible a ready recognition of certain organisms and permits an easy distinction of this relatively small group of bacteria from the countless other forms found in nature. At the same time it has led to confusion, at times serious, in a failure to distinguish virulent and avirulent forms within the group, as will be brought out in more detail below. Consequently this staining peculiarity has assumed considerable practical as well as theoretical importance.

It is usual to consider certain other tinctorial peculiarities, namely alcohol-fastness and alkali-fastness, that is, resistance to destaining by alcohol and alkali, as related phenomena. Organisms displaying one type of fastness usually show the others also, although the attempt has been made to make certain fine distinctions in this field the basis of distinguishing organisms within the acid-fast group. It is to be pointed out that the acid-fast organisms are also Gram positive. The phenomena may be more than coincidental, for some investigators have related both to the permeability of the cells concerned to certain chemical reagents; in the case of the acid fast organisms the permeability to acid, alkali and alcohol is concerned, and in that of the Gram-positive organisms, to alcohol, after the cell wall has been modified by the action of iodin (Benians).<sup>1</sup> However, this view is not universally accepted.

The entire organism is not always equally acid-resistant. There are the familiar "beaded forms" of tubercle and leprosy bacilli, in which the discontinuity of acid-fastness within single long bacilli may give the picture of strings of acid-fast cocci. Although such beaded forms are often considered degeneration forms,

<sup>1</sup> For a discussion of Gram staining see Wells' Chemical Pathology, 4th Ed., 1920, p. 108.

perhaps the result of resistance on the part of the host<sup>2</sup> and therefore of chronicity of disease, caution is necessary in interpretation, as similar pictures may be produced in fixation by overheating.

Furthermore it has been shown by Much<sup>3</sup> that by proper staining methods other evidences of non-homogeneity can be detected in tubercle bacilli. He claimed to find in many cases of bovine tuberculosis and bone and gland tuberculosis in man, in which acid-fast bacilli could not be found, granular masses and granular bacilli stainable by Gram's method. He came to believe there were three states in which the tubercle bacillus occurred, all virulent, the acid-fast form and Gram positive non-acid-fast granules and granular bacilli. He inclined to the view that the granular forms might represent developmental stages. This idea has met no general acceptance.

Bergel,<sup>4</sup> who, like many others, attributes to the lymphocyte an important rôle in tissue resistance to tuberculosis, considers the Much forms the result of lipolytic action of the small mononuclears upon the waxy shell of the organisms. Bergel pictures the tubercle bacillus as consisting of a series of protein nuclei, perhaps joined by protein threads, surrounded each by a waxy layer, the whole series being again inclosed in a lipoid inter-layer, clothed in turn by a mantle of wax.

It must not be forgotten that the property of acid-fastness is not confined to the so-called acid-fast bacteria. The spores of non-acid-fast bacteria may be acid-fast. Parts of multicellular organisms also have been found to resist decolorization by acids, for instance, the eggs of certain taenia (see Helbing below). Probably the factors concerned in the property are the same in most cases.

A consideration of chemical and morphological peculiarities which might be factors in conferring acid-fastness will be given in the pages to follow.

<sup>2</sup> The finding of beaded forms has been made a factor in the consideration of discharge of lepers in certain leprosy detention sanatoria in Hawaii. (Personal communication to the author by Dr. J. T. MacDonald.)

<sup>3</sup> Beitr. klin. Tuberk., 1907 (8), 85.

<sup>4</sup> Beitr. klin. Tuberk., 1917 (38), 95.

A very large literature on acid-fastness has accumulated since the original discoveries by Neisser, Koch and Ehrlich. Much of this, however, represents repetition, merely with confirmation of a few findings. The underlying principle of acid-fastness is still unknown. Perhaps it would be better to say that a number of underlying principles are known, but that at present they are uncorrelated.

It was Neisser's keen observation which led to the first distinction of an organism as acid-fast. He noted the property in the rods discovered in lepra cells by Hansen, and accorded to these rods their correct position in the world of living beings, as bacteria. Koch, who succeeded where others failed in the isolation of the cause of tuberculosis, attributed his success partly to the use in staining of an agent which modified permeability, potassium hydroxide. The same factor played a rôle when, in counterstaining with vesuvin, the bacilli retained their methylene blue stain and failed to take the counterstain. Tissue cells, tissue detritus and other bacteria, lost their methylene blue stain and counterstained brown when sections of tuberculous tissues, stained at 40° with slightly alkaline methylene blue, were washed with the dye vesuvin. Ehrlich extended Koch's observations and placed on record a method which has formed the basis for subsequent techniques in staining organisms of this group. He used strong mineral acids as decolorizers preceding counterstaining, and was the first to accord extensive consideration to the phenomenon of acid-fastness. His views will be referred to below in the consideration of theories on its nature.

Since Ehrlich's classic investigation a great variety of methods for distinguishing bacteria on the basis of acid-fastness have been proposed. The best known of these are the familiar Ziehl-Neelsen and Gabbet techniques. The fundamental principle underlying all of them is the resistance of a difficultly stainable organism, when once stained, to decolorization. Probably many dyes are suitable for the original staining, just as many might be used, and actually are used, in counterstaining for the purpose of bringing out other bacteria and tissue material. The attempt has in general been made to select colors which will bring out the maximum amount of contrast between the acid-fast bacillus and

other elements, and thereby facilitate rapid diagnosis. Red-blue and red-yellow combinations have been the most popular.<sup>5</sup>

A number of methods have been devised for distinguishing between true tubercle bacilli and avirulent acid-fast microorganisms. On the whole these have not proved very reliable. The distinction is obviously one of great clinical importance. Those methods which have been devised depend generally upon the greater resistance of tubercle bacilli to destaining. Some of the earlier investigators reported that the smegma bacillus was acid-fast but not acid-alcohol fast. Leyden<sup>6</sup> and Grethe<sup>7</sup> believed that it could be distinguished from the tubercle bacillus in this way. Certainly there are numerous exceptions to this view, if the exceptions are not the rule. Many others have found the smegma bacillus less resistant to prolonged acid decolorization than the tubercle bacillus. I recall a specimen of urine with scores of acid-fast bacilli to the  $\frac{1}{2}$  oil immersion field in smears stained with carbol fuchsin and destained with 70 per cent alcohol containing 3 per cent hydrochloric acid. The bacilli concerned, however, failed to retain the fuchsin coloration when subjected to overnight treatment with 25 per cent nitric acid. They were also avirulent for guinea pigs. Tubercle bacilli retain the stain even under the conditions cited. I have found, however, that smegma bacilli under prolonged cultivation in the laboratory on glycerol-peptone agar, become practically as acid-fast as tubercle bacilli. According to Cépéde<sup>8</sup> if, before staining, the smear is treated five to ten minutes with 5 per cent alcoholic sodium hydroxide the smegma bacillus will lose its acid-fastness while the tubercle bacillus will not. Cépéde claimed that the alkali treatment removed the fat from the smegma bacillus, but did not deprive the tubercle bacillus of its wax. Dold,<sup>9</sup> however, believed alkali-fastness a

<sup>5</sup> For a compilation of the methods of staining acid-fast microorganisms, the reader is referred to Hetzel (*Arch. wissenschaftl. prakt. Tierheilkundes*, 1921 (47), 109).

<sup>6</sup> Verein. inn. Med. Berlin, Mar. 30, 1896. Abstr. Deut. med. Woch., 1896, 22, 121 V.

<sup>7</sup> Fortschritte d. Med., 1896 (14), 329.

<sup>8</sup> Compt. Rend. Acad. Sci., 1918 (166), 357.

<sup>9</sup> Arb. kaiserl. Gesundh., 1911 (36), 433.

property of the entire group and no basis for distinction within the group. He found that a number of acid-fast microorganisms tested by the method of Gasis (essentially staining with warm, aqueous eosin containing a trace of mercuric chloride, and de-staining with 0.5 per cent sodium hydrate) retained their eosin stain.

Relationship between age of bacillus and acid-fastness has been recorded several times. Marmorek<sup>10</sup> found that young cultures, or the younger portions of old cultures, contained numerous organisms that were not acid-fast. He distinguished two phases of growth on fluid media, a thin skin and a thick skin stage. The thin skin, or early growth, which resembled a cholesterol film on serum, contained numerous non-acid-fast bacilli, a smear preparation giving the impression of a contaminated culture. That it was not contaminated, however, was proved by the fact that the older, thick skin cultures no longer contained the blue staining bacilli. Very aged cultures on the other hand did contain some non-acid-fast forms. Marmorek believed that in the young bacilli sufficient time had not elapsed for the elaboration of protective substances in the capsule, which he assumed to be of waxy or fatty nature. The partial or complete loss of acid-fastness occasionally observed in old bacilli he attributed to the presence of some metabolite in the medium affecting the permeability of the cell. He noted, however, that the artificial introduction of tuberculin in the medium of growing cultures did not lead to any increase in the non-acid-fast forms.

Klein<sup>11</sup> also found that young bacilli taken from the edges of colonies were in numerous instances non-acid-fast toward 33 per cent nitric acid. Like Marmorek he attributed the lack of acid-fastness to the youth and attendant deficiency of the bacilli in those chemical constituents responsible for acid-fastness. His findings were made with cultures on horse serum and led him to believe that the development of acid-fastness did not depend on a high fat content of the medium.

<sup>10</sup> Zeit. f. Tuberk., 1900 (1), 444.

<sup>11</sup> Centr. f. Bakt. Parasit., 1900 (28), (I), 111.

Suyenaga<sup>12</sup> has confirmed these findings, using a rapidly growing bacillus, colonies of which could be examined after twenty-four to forty-eight hours. He noted that rapid transfer of cultures, with inoculation of material from the edges of young colonies, did not lead to the production of a strain deficient in acid-fastness. In other words acid-fastness depended upon maturity, rather than individual persisting differences in microorganisms. In his second communication he reported the finding of non-acid-fast forms also in virulent cultures. In a study of several acid-fast forms, including avian tuberele bacilli, frog, "leprosy" and smegma bacilli, he found that those forms naturally least acid-fast (the frog and avian strains used) were more susceptible to the bactericidal and inhibitory action of gentian violet and methylene blue, than the others. Suyenaga also confirmed the finding of Wherry, which will be referred to below, that bacilli grown on media low in nutritive value were less resistant to acid decolorization than those which had developed on the usual media.

Cantacuzene<sup>13</sup> found that young cultures of the timothy bacillus growing on gelatin, glycerolated or not, exhibited the same phenomenon. Many non-acid-fast forms were seen in two to three day cultures, while acid-fastness was general at fifteen days.

Numerous theories have been put forward to account for the phenomenon of acid-fastness. Ehrlich<sup>14</sup> advanced the hypothesis that it was the result of a low degree of permeability of the bacterial envelope. He pointed out that the tubercle bacillus required special methods in staining, such as the use of alkali, as recommended by Koch, and the application of heat, unnecessary in the case of other bacteria. Bacteria thus difficult to stain were also difficult to destain, at least with acids. He attempted to draw a practical application from these facts suggesting that alkaline rather than acid disinfectants would be most efficient.

Considerable support has been found for Ehrlich's conception that acid fastness is due to difficulty in permeating the capsule.

<sup>12</sup> Amer. Review Tuberc., 1919 (3), 473; 1920 (4), 526.

<sup>13</sup> Compt. Rend. Soc. Biol., 1905 (59), 384.

<sup>14</sup> Deut. med. Woch., 1882, (8), 269.

Koch<sup>15</sup> was perhaps the first to point out that disintegration of the organism deprived it of acid-fastness. In the course of his attempts to devise a tuberculin containing all of the constituents of the bacillus in readily absorbable form he ground dried bacilli in an agate mortar to a state of extreme fineness. The powder yielded an opalescent suspension in salt solution containing practically no intact bacilli. Stained specimens destained readily with acid.

Benians<sup>16</sup> found that the substance of tubercle bacilli crushed in a mortar or between glass slides, unlike the intact microorganism, could be stained easily and was to a considerable extent non-acid-fast. In the ground mass only bacilli morphologically intact retain the stain against acid. This observation has been confirmed in this laboratory by Hope Sherman.<sup>17</sup> She called attention to the fact, long known but frequently lost sight of, that an ordinary culture of tubercle bacilli contains, besides the bacteria themselves, an extrabacterial substance, playing a rôle in staining reactions.

Numerous attempts have been made to modify the permeability of the bacterial cell by gentler means, particularly by the action of chemicals. Alkalies have frequently been used in this connection. Considerable discrepancy occurs in the recorded results on the effect of treatment of smears with moderately concentrated soda or potash prior to staining. Certainly the bacillus shows considerable resistance to alkalies. In my experience prolonged treatment with 5 to 10 per cent sodium hydrate does not modify acid-fastness. With concentrations of 20 to 25 per cent, however, considerable effect is noted, many blue staining rods and a good deal of blue staining detritus being observed in the alkali treated smear, after the usual Ziehl-Neelsen technique.

Deycke and Much<sup>18</sup> stated that suspension of tubercle bacilli in brain emulsion resulted in a loss of acid-fastness, which they were led by further experimentation to attribute to the lytic action of lecithin and neurine. Complete bacteriolysis, however, was

<sup>15</sup> Deut. med. Woch., 1897 (23), 209.

<sup>16</sup> Jour. Path. Bact., 1912 (17), 199.

<sup>17</sup> Jour. Infect. Dis., 1913 (12), 249.

<sup>18</sup> Münch. med. Woch., 1909 (56), 1985.

by no means general with brain extracts, but a constant loss of the property of acid-fastness with granular degeneration of the bacilli was noted upon treatment with 10 per cent lecithin emulsion. The granules took the Gram stain. These were considered similar to the "Much granules" described by Much<sup>3</sup> as a special, virulent form of the bacillus, occurring in certain lesions, stainable by Gram's method but not by Ziehl's, and capable of reproducing typical, virulent acid-fast bacilli after cultivation on artificial media. In the experiments of Deycke and Much a similar granular degeneration of tubercle bacilli with loss of acid-fastness could be observed on injection of tubercle bacilli into the peritoneal cavity of a guinea pig, especially if the guinea pig already had tuberculosis, in which case practically complete lysis was observed. Lysis, more nearly complete than with lecithin, was caused by the action of neurine. These observations have been disputed, particularly by Uhlenhuth<sup>19</sup> and by Löwenstein,<sup>20</sup> the latter repeated the experiments with neurine and was unable to confirm the results of Deycke and Much, which he attributed to errors in technique.

Aronson<sup>21</sup> in the main agreed with Löwenstein, finding that bacilli were left acid-fast after the action of 25 per cent neurine at 37° for days. Complete solution of the bacilli occurred, however, at 56°. Jensen and Rabinowitsch<sup>22</sup> attributed the effect of neurine solely to its alkalinity.

Bontemps<sup>23</sup> was unable to demonstrate complete solution of tubercle bacilli through the action of neurine at 37°, but found that at 56° solution of certain substances, particularly lipins, occurred. Complete solution, such as takes place in some bacteria in antiformin, did not occur. The degree of solution caused was greater than that observed with other alkalies, including 25 per cent soda and potash, ammonia, trimethylamine and tetramethyl ammonium hydrate. Bontemps also found that certain acids, particularly tartaric, citric and lactic, in 50 per cent aque-

<sup>19</sup> Quoted by Deyke and Much.<sup>18</sup>

<sup>20</sup> Cent. f. Bakt., 1910 (53), 541.

<sup>21</sup> Berl. klin. Woch., 1910 (47), 1617.

<sup>22</sup> Cent. f. Bakt., 1910 (54), 454.

<sup>23</sup> Zeit. f. Immunität., 1912 (15), 436.

ous solution, caused considerable dissolution, with loss of acid-fastness. Gram-positive granules were present. Formic, acetic, butyric and valerianic acids caused little change other than some loss of acid-fastness. Pepsin hydrochloric acid caused solution of tubercle bacilli, only amorphous masses being left; a considerable amount of liberated lipin accumulated on the surface of the mixture. Trypsin had little or no effect.

Porter,<sup>24</sup> following the prevalent notion that the impermeability of the capsule was due to a concentration of the lipins of the organism within the envelope, is one of several who have tested the effect of lipases and esterases upon living organisms of the acid-fast group. Of tissues examined, human skin was the most bactericidal, it was also exceptionally rich in esterases. Lymph gland material, containing a moderate concentration of esterase, was also strongly bactericidal. Dead bacilli from lipolytic mixtures for the most part stained perfectly with the Ziehl-Neelsen method, although a certain percentage lost their acid-fast property. According to Porter proteolytic ferments affect the staining properties of acid-fast bacilli much more profoundly than do lipolytic ferments.

According to Salimbeni,<sup>25</sup> trichlorhydrin deprives tubercle bacilli of their acid-fastness in a few minutes treatment, the organisms becoming granular or losing their form altogether. The product stains with the methylene blue in the Ziehl-Neelsen technique.

The investigations just cited deal with methods more or less harsh, by which the limiting membrane has been supposedly disrupted or made more permeable by the action of chemicals, and the property of acid-fastness has been lost. Needless to say viability was destroyed in most instances.

Another series of investigations has dealt with modification of acid-fastness by varying the medium of growth. Very soon after the discovery of the tubercle bacillus and demonstration of its acid-fastness the impression became prevalent that acid-fastness was in some way associated with the fatty or waxy

<sup>24</sup> Jour. Hyg., 1917 (16), 55.

<sup>25</sup> Compt. Rend. Acad. Sci., 1912 (155), 368.

nature of the microorganism. Bienstock<sup>26</sup> went so far as to state that other bacteria, non-acid-fast under ordinary conditions, including a "protein bacillus" from feces, *B. subtilis*, *B. pyocyaneus*, and *B. typhosus*, could be made to take on the acid-fast property by growing them upon fat-rich media, as a butter-agar-gelatin. Furthermore, merely rubbing the non-acid-fast bacilli with butter rendered them somewhat resistant to acid decolorization. Essentially similar results were obtained by Gottstein,<sup>27</sup> who found, however, that the resistance to destaining agents on the part of these bacilli rendered acid-fast artificially, was, as compared with the tubercle bacillus, low. Treatment with boiling alcoholic 2.5 per cent potash removed their acid-fastness, although ineffective toward tubercle bacilli, but they were quite resistant to chloroform. It has been reported also that the property may be acquired to a certain extent by growth in fibrinous exudates and in blood.<sup>28</sup>

Conversely, it has been claimed by several investigators that by modification of the culture medium acid-fastness may be reduced. Ferran<sup>29</sup> found that suppression of the peptone and gradual decrease of the glycerol and glucose were especially effective in this regard. He claimed that ultimately a rapidly growing, non-acid-fast saprophyte developed. The primitive virulent, acid-fast type could be recovered on intraperitoneal injection into guinea pigs. These observations could not be confirmed by Ramond and Ravant.<sup>30</sup>

Wherry,<sup>31</sup> using a saprophytic descendant of a tubercle bacillus (isolated twenty-five years before), which would grow without glycerol, found that on media not suitable for good growth, where carbon was supplied by various sugars and methyl, ethyl, propyl and isobutyl alcohol, and nitrogen in the form of a simple ammonium salt, there appeared coccoid chains, spore-like bodies and many non-acid-fast rods. He believed that conditions unfavorable

<sup>26</sup> Fortschritte d. Med., 1886 (4), 193.

<sup>27</sup> Fortschritte d. Med., 1886 (4), 252.

<sup>28</sup> See Philibert, Les Pseudo-bacilles acido-résistants. Paris, 1908.

<sup>29</sup> Compt. Rend. Acad. Sci., 1897 (126), 1555.

<sup>30</sup> Progr. méd., 1900 (12), 429.

<sup>31</sup> Cent. f. Bakt., 1913 (70), 115; Jour. Infect. Dis., 1913 (13), 144.

to the synthesis of fats were responsible for the loss of acid-fastness. Kendall<sup>32</sup> confirmed these observations for early stages of cultures on synthetic media, but found that after three weeks even on very simple media all bacilli were acid-fast.

Miller<sup>33</sup> found that tubercle bacilli grown upon media rich in glycerides of oleic acid, such as sperm and olive oil, were exceptionally beaded when stained with carbol fuchsin. By analogy with the condition occurring when an aqueous solution of fuchsin is shaken with oleic acid, in which the oleic acid takes out the color, itself acquiring a deep magenta stain, Miller concluded that certain granules in the tubercle bacilli of his experiments had taken up the esters of oleic acid of his media and from them free oleic acid had been liberated in the granules which then took the stain in high concentration at the expense of the rest of the organism.

Dostal<sup>34</sup> has described what he terms the glucoside form of tubercle bacillus, in which the biology of the bacillus is so modified by repeated passage on media containing 5 to 10 per cent saponin that luxuriant growth occurs, the organisms finally becoming neither alcohol- nor acid-fast. This has not been confirmed.

As pointed out above, the acid-fastness of the tubercle bacillus has been considered by many to be the result of disposition of certain of its constituents in the form of a difficultly permeable envelope. Others, less concerned with the morphological arrangement of the material, have attempted to prove by chemical isolation that certain fractions of the microorganisms were responsible for acid-fastness.

Since the tubercle bacillus is perhaps the richest in fatty materials of all organisms known, and since other members of the acid-fast group have in a vague way been known also to be fat-rich, a relation has long been assumed between the lipin of the tubercle bacillus and its strong retention of stain. As mentioned above the impression was prevalent four years after the discovery of the germ that it owed its acid-fastness to its waxy nature, and several observers reported conferring acid-fastness on bacteria other than the acid-fasts by growth on fat-rich media. Koch<sup>15</sup> records that

<sup>32</sup> Jour. Infect. Dis., 1914 (15), 428.

<sup>33</sup> Jour. Path. and Bact., 1916 (21), 41.

<sup>34</sup> Frankfurter Zeit. Path., 1916 (19), 198.

he had learned with Proskauer that there were two peculiar substances in the tubercle bacillus related to the fats, varying in their solubility in alcohol, one being soluble only in boiling absolute alcohol or in ether and being difficultly saponifiable. These were both acid- and alcohol-fast, and were to be considered the carriers of the peculiar staining property. Klebs,<sup>35</sup> also, definitely attributed acid-fastness to the fat content, defatted bacilli according to this observer not possessing it. Aronson,<sup>36</sup> one of the earliest observers to extract large amounts of fatty material from the bacillus and to call attention to its waxy nature, considered this wax the carrier of acid-fastness. He found that the stained wax resisted acid decolorization. The fat from diphtheria bacilli he found was non-acid-fast. He made the observation, however, that after long continued extraction with fat solvents the bacilli are still acid-fast. If, however, the final extractions were made with a mixture of alcohol and ether containing 1 per cent hydrochloric acid the property was removed. In trichlorethylene he found a reagent capable of removing the wax all at once. Three grams of bacilli from which the moisture had been expressed between filter papers were rubbed up in this solvent and exposed to its action in a sealed tube at 37° for two days; at the end of this time the bacilli consisted of indistinctly outlined rods, quite non-acid-fast. The Much granules were no longer stainable; these, being soluble in a fat solvent, could therefore not be considered protein. The action of the trichlorethylene was not an effect of hydrochloric acid dissociated from it, for it took place equally well with trichlorethylene treated with soda solution. Aronson made the interesting observation that streptococci and staphylococci treated with trichlorethylene retained their Gram-positive character, while tubercle bacilli did not; different substances were apparently responsible for the staining property in the two forms.

Bulloch and Macleod<sup>37</sup> confirmed Aronson's observation on the impossibility of rendering tubercle bacilli non-acid-fast by the use of fat solvents alone. For more complete extractions they

<sup>35</sup> Cent. f. Bakt., 1896 (20), 488.

<sup>36</sup> Berl. klin. Woch., 1898 (35), 484; 1910 (47), 1617.

<sup>37</sup> Jour. Hyg., 1904 (4), 1.

used the method of Aronson, adding hydrochloric acid to the methylated spirit used. The waxy precipitate which separated out from Aronson's mixture on cooling was strongly acid fast. After staining with hot carbol fuchsin for five minutes it retained its stain when subjected to eight days immersion in 25 per cent sulphuric acid. From bacilli which had been treated with methylated spirit but were still acid-fast, they separated by prolonged boiling with alcoholic potash a substance soluble in ether and ligroin, which was shown to be a pure alcohol, which was strongly acid-fast when spread on glass slides. Fatty acids isolated from the bacillus were non-acid-fast.

Camus and Pagniez<sup>38</sup> used the following simple method of testing acid-fastness. A drop of the material in question, such as a fat in solution or melted fat, was placed on a filter paper and the paper stained and decolorized by the method of Ziehl. They found that fatty acids of high molecular weight ( $C_{18}$ ,  $C_{16}$ ) were acid-fast while those of low molecular weight ( $C_3$ ) were not. They felt the difference might be more apparent than real, however, because of the water solubility of the lower members of the fatty acid series. Neutral fat was not acid-fast while rancid fat was. They attributed the acid-fastness of the tubercle bacillus to its free fatty acid content. They refute Ciaccio's<sup>39</sup> doubt on the rôle of fatty substances, which was based on the fact that defatted bacilli, failing to stain with Sudan III, are still acid-fast, by emphasizing the great difficulty in getting complete extraction of fat. They also call attention to the claim of Babes and Cantacuzene<sup>40</sup> that tubercle bacilli could be completely defatted and at the same time rendered non-acid-fast by successive treatments with methyl alcohol and petroleum ether.

Fontes<sup>41</sup> extracted bacilli successively with xylene, alcohol, ether and chloroform. The extracted bacilli remained for the most part acid-fast, although granular. Absolute alcohol added to the xylene extract precipitated a waxy substance partly soluble in ether and wholly so in chloroform, which microscopically consisted of small refractile granules which were acid-fast.

<sup>38</sup> Presse Médicale, 1907 (15), 65.

<sup>39</sup> Compt. Rend. Soc. Biol., 1906 (60), 585.

<sup>40</sup> Ann. Inst. Pasteur, 1905 (19), 699.

<sup>41</sup> Cent. f. Bakt., 1909 (49), 317.

Tamura<sup>42</sup> confirmed the point made by Bulloch and Macleod that the alcohol of high molecular weight obtained from the tubercle bacillus, which in the purified state was termed "mykol" by Tamura, was acid-fast, whether tested on a microscopic slide or in quantity in the test tube. He also investigated the combining power of the alcohol for fuchsin. His results indicated that the union was one of physical absorption, no molecular relation holding, for 0.2922 gram of mykol on treatment with 5 per cent fuchsin yielded 0.3055 gram of colored substance, or 6.09 per cent increase, while with 8 per cent fuchsin the increase amounted to 8.37 per cent. The mykol was also alkali-fast, Gram positive and stainable by the method of Much. The mykol esters, in which form the mykol exists in the bacillus, behaved similarly to the free alcohol. Tamura believed it was not necessary to adduce the idea of a difficultly permeable membrane to explain the staining reactions of the acid-fast bacteria.

Goris and Liot,<sup>43</sup> whose article may be consulted for a review of the literature, also found mykol to be acid-fast. The method used was similar to that of Camus and Pagniez. Paper strips were impregnated with a chloroform solution of various lipin fractions, dried, soaked five minutes in hot carbol fuchsin solution, destained in 25 per cent sulphuric acid, then in alcohol at 60°, and finally washed in water. The mykol was found to be the fraction most strongly acid-fast. The solid fatty acids were moderately so. Hyalinol, a brittle, elastic lipoid melting at 175°, soluble in chloroform, but insoluble in acetone and ether, which made up 2.5 per cent of the total lipin, was not acid-fast.

As has been indicated, a majority of men who have concerned themselves with the problem have considered the acid-fastness of the bacillus a function of its high fat content. Other explanations, however, have not been wanting. Several investigators, impressed by the fact that bacilli leached out as thoroughly as possible with fat solvents remained acid-fast, have looked in other fractions of the bacillus than the lipin for the carrier of the staining peculiarity. Hammerschlag,<sup>44</sup> who made the earliest

<sup>42</sup> Zeit. physiol. Chem., 1913 (87), 85.

<sup>43</sup> Ann. Inst. Pasteur, 1920 (34), 497.

<sup>44</sup> Zent. klin. Med., 1891 (12), 9.

extensive chemical investigation of the tubercle bacillus, considered the residue left after his fat extractions a protein-cellulose combination. Extraction of the protein by sodium hydrate destroyed acid-fastness, but the protein taken out, while readily stainable, was not acid-fast. He believed, therefore, that acid-fastness was a property inherent in the protein-cellulose combination of the intact cell.

Ruppel<sup>45</sup> considered the residue left after fat extraction "proteinoid," and compared it to chitin. Helbing,<sup>46</sup> too, compared the acid-fastness of the tubercle bacillus with that of the shell of certain taenia eggs known to contain chitin, and suggested chitin as responsible for acid-fastness.

Gasis<sup>47</sup> believed that acid-fastness is due, not to a fat, nor a substance analogous to chitin, nor to the physical state of the protoplasm, but to a protein. He pointed out that the tubercle bacillus would stain, not only with the basic aniline dyes, as had been generally supposed, but also with the acid dye eosin. He noted also in staining certain tissue preparations that tubercle bacilli and the eosinophilic granules of leucocytes stained the same. He believed that they were of similar composition, and concluded that the bacilli owed their staining properties to what he considered the granules to be, protein.

Grimme<sup>48</sup> had already come to the conclusion that protein was not responsible for acid-fastness, in view of the fact that trypsin was without effect upon the staining power of the bacillus. Two days action of pepsin-hydrochloric acid did destroy the acid fast property of the bacillus, but Grimme was able to show that treatment with the same strength of acid for the same time was equally effective. He concluded on this basis that the substance responsible was not a fat, because fats should not yield thus to treatment with hydrochloric acid, and the fat which he isolated from the bacillus was not very acid-fast. He believed acid-fastness to be due to a specific substance in the body of the bacillus, not a protein and not a fat, but beyond this he was unwilling to commit

<sup>45</sup> Zeit. physiol. Chem., 1898 (26), 218.

<sup>46</sup> Deut. med. Woch., 1900 (26), 133.

<sup>47</sup> Cent. f. Bakt., 1909 (50), 111.

<sup>48</sup> Cent. f. Bakt., 1902 (32), 161.

himself, other than to state that the substance was soluble in reagents with as little in common as 80 per cent alcohol, xylene, ether and 0.5 per cent hydrochloric acid, and was destroyed by Javel water.

Auclair and Paris,<sup>49</sup> familiar with the ideas prevalent at their time concerning acid-fastness, and equipped with experimental evidence of their own, put forth the thesis that not one, but all of the chief constituents of the bacillus, were acid-fast, and that the acid-fastness of the germ was the sum of the acid-fastness of its constituent parts. Four months extraction with fat solvents, until no more lipin could be obtained, left the bacilli still acid-fast. Extraction with hot 2 to 10 per cent potash, while it removed an acid-fast protein, left an acid-fast residue. This residue, on the basis of the blue color it developed on treatment with sulphuric acid and iodine, was considered to be a hydro-cellulose. Thus acid-fastness appeared to be the sum of the stain-retaining powers of fat, protein and carbohydrate.

Schlossberger<sup>50</sup> pointed out that in general the saprophytic acid-fast bacilli have been considered less acid-fast than the pathogenic forms, and he would agree, although stating that there are many exceptions in both columns. He noted that passage strains of acid-fast saprophytes, for instance, butter bacilli which had been inoculated into guinea pigs and recovered, displayed more resistance to decolorization with nitric acid than before passage. This property was accompanied by increased virulence and slower growth on artificial media. He stated that he had no reason for believing that the saprophytic acid-fast bacilli contained less extractable fat or wax than true tubercle bacilli, but surmised that there might be some difference in the distribution of the wax in the inner parts of the protoplasm, with which the staining property might be concerned.

From the above summary it will be seen that there is considerable conflict in the views of those who have attempted to explain the acid-fastness of tubercle bacilli. Ehrlich's original hypothesis of an acid impermeable membrane has been modified or discarded

<sup>49</sup> Arch. d. méd. exp., 1907 (19), 129.

<sup>50</sup> Beit. Klin. Tuberk., 1922 (50), 144.

altogether by numerous succeeding investigators, who have demonstrated acid-fastness in a given chemical fraction, wax, protein or cellulose-like substance, and been inclined to attribute the staining peculiarity of the microorganism in its entirety to that particular constituent. Stress has been laid upon the wax and other lipins by the majority of observers.

Several irrefutable facts stand out as the result of the investigations to date. The most conspicuous of these are perhaps the following:

1. Disintegration of the cell destroys acid-fastness.
2. Extraction of the cell body with fat solvents until no more lipin is removed, leaves the bacilli acid-fast, provided they have not been mechanically disintegrated, for example, by trituration, in the process.
3. The wax of the bacillus is acid-fast.

Exceptions have been taken to these statements, especially to the claim that extraction of the bacilli with fat solvents is incapable of removing the property of resisting acid decolorization. The great majority of observers find this to be true, however, and it is to be pointed out that a number of men who have claimed loss of acid-fastness with complete defatting have combined with their extractives some more active chemical, as for example hydrochloric acid. Others have certainly destroyed the form of the bacillus mechanically by trituration in their solvents, or by subjection to prolonged ebullition in the high boiling fat solvents, like xylene.

I<sup>51</sup> have attempted to reconcile the two chief conflicting explanations of acid-fastness, viz., that based on the conception of a relatively impermeable membrane, and that which credited the acid-fastness of the whole bacillus to its content of an acid-fast wax. It was found that dehydration with boiling absolute alcohol and attendant removal of the alcohol soluble fats, followed by prolonged treatment with such a good lipin solvent as medium boiling petroleum ether, left the bacilli acid-fast. This was equally true of tubercle bacilli of human, bovine and avian types, and of frog and turtle bacilli, the leprosy bacillus of Duval, smegma bacilli and grass bacilli. However, such bacilli, although thoroughly leached with petroleum ether until no more lipin would

<sup>51</sup> Amer. Rev. Tuberc., 1922, (6), 642.

come out, were not lipin-free. It will be recalled that Aronson and Bulloch and Macleod had combined an acid with their fat solvents to obtain complete extraction. I divided this procedure, and after defatting bacilli as completely as possible with alcohol and petroleum ether, treated them with normal hydrochloric acid at 37° for forty-eight hours and again extracted with alcohol and petroleum ether. From 1 to 8 per cent more lipin came out. Acid-fastness and integrity of the bacillary cell were lost at the same time. *B. subtilis*, which is non-acid-fast, and which has a low total lipin content, was found to contain lipin similarly firmly bound, and in the same amount as is found on the average in acid-fast bacilli, about 5 per cent of the dry weight.

A summary of the results of the investigation is given in the following table:

| BACILLUS                               |      | LIPIN REMOVED BY PETROLEUM ETHER BEFORE ACID TREATMENT, IN PER CENT OF DRY WEIGHT OF BACILLUS | ACID FASTNESS OF DEFATTED MATERIAL BEFORE ACID TREATMENT | PRESERVATION OF BACILLARY FORM OF DEFATTED BACILLI | ACID FASTNESS OF DEFATTED BACILLI AFTER THROUGH GRINDING BETWEEN GLASS SLIDES | ADDITIONAL LIPIN REMOVED BY PETROLEUM ETHER AFTER ACID TREATMENT, IN PER CENT OF DRY WEIGHT OF BACILLUS | LIPIN REMOVABLE AFTER ACID TREATMENT, IN PER CENT OF TOTAL LIPIN | ACID FASTNESS AFTER ACID TREATMENT | PRESERVATION OF BACILLARY FORM AFTER ACID TREATMENT |
|--|------|---|--|--|---|---|--|------------------------------------|---|
| H 37 human type tubercle bacillus..    | 22.7 | +   | +  | 0  | 7.8   | 25.6  | 0  | 0                                  |   |
| B 1 bovine type tubercle bacillus..    | 22.3 | +   | +  | 0  | 3.8   | 14.5  | 0  | 0                                  |   |
| A 1 avian type tubercle bacillus ..... | 11.0 | +   | +  | 0  | 0.8   | 6.8   | 0  | 0                                  |   |
| Leprosy bacillus of Duval.....         | 9.0  | ++  | +  | 0  | 4.1   | 29.7  | 0  | 0                                  |   |
| Turtle bacillus.....                   | 36.3 | ++  | +  | 0  | 4.8   | 11.6  | 0  | 0                                  |   |
| Frog bacillus.....                     | 37.1 | ++  | +  | 0  | 4.1   | 10.0  | 0  | 0                                  |   |
| Smegma bacillus....                    | 35.6 | ++  | +  | 0  | 2.3   | 6.1   | 0  | 0                                  |   |
| Grass bacillus.....                    | 23.4 | ++  | +  | 0  | 2.3   | 9.0   | 0  | 0                                  |   |
| <i>B. subtilis</i> .....               | 4.4  | 0   | +  | 0  | 5.1   | 53.6  | 0  | Spores left intact                 |   |

The great variance in the amount of firmly bound lipin present, calculated in per cent of the dry weight, was believed to indicate that the union was a physical, not a chemical one. The lipin removed appeared to be the same in all cases. It was acid-fast, but fully as much so in the case of that removed from *B. subtilis* as in that from the acid-fast bacilli. It was soluble in ether, chloroform, petroleum ether, and benzene, slightly soluble in hot alcohol, insoluble in cold alcohol and insoluble in acetone. It was phosphorus-free. The melting point was between 42° and 48°. These are the characteristics of the wax which makes up the larger part of the lipin of the tubercle bacillus. Goris has considered this to be mykol laurate, mykol being the solid aleohol isolated by many investigators and named by Tamura. Bulloch and Macleod had found that the bacilli left after prolonged extraction with methyl alcohol, which were still acid-fast, were a good source for the preparation of the characteristic solid alcohol. By long boiling of these bacilli with alcoholic potash they separated a substance soluble in ether and ligroin, which chemical examination proved to be a pure alcohol. I believe the lipin of the firm lipin-protein union to be an ester of this substance, and present not in true chemical union with protein analogous to that in such conjugated proteins as nucleoproteins and glycoproteins, but probably in emulsion in a protein substrate. It probably plays a rôle in acid-fastness, not through its inherent resistance to de-staining, but through the manner of its disposition in the cell. The finding of similar lipin-protein in *B. subtilis*, which is non-acid-fast, requires the conception of a different manner of disposition in the two types of microorganism. This conclusion is supported by the fact that extracted bacilli may be deprived of their acid-fastness by the simplest of mechanical means. Whereas prolonged vigorous grinding is necessary to deprive intact bacilli of their acid-fastness, as in the early experiments of Koch and later ones of Benians and Sherman, less severe pressure between glass slides is sufficient to turn defatted bacilli into a formless mass which takes the blue, not the fuchsin, in the method of Ziehl and Neelsen. No chemical change, probably, has taken place. If chemical affinity rather than manner of disposition is to account for acid-fastness, then in the instance just

cited loss of acid-fastness may be explained only by a finer emulsification of the acid-fast constituent. That this is not impossible is indicated by analogy with the well known experiments of Martin Fischer<sup>52</sup> in connection with the staining of fats in fatty degeneration of tissues, which indicate that the unstainability of the fats in normal cells depends upon their perfect emulsification. Fischer and the author have found, moreover, that stained tubercle bacillus wax may appear to lose some of its color upon emulsification in sodium oleate or alkali protein suspension.

<sup>52</sup> Fats and Fatty Degeneration, Wiley and Sons, New York, 1917.

## CHAPTER IV

### OTHER ACID-FAST BACTERIA

The phenomenon of acid-fastness, that is, the resistance of stained bacteria to acid decolorization, was first noted, a year before the discovery of the tubercle bacillus, by Neisser<sup>1</sup> in his application of Weigert's staining methods in the investigation of Hansen's leprosy bacillus. Neisser found that fuchsin stained the organism only faintly in neutral solution, but more readily when a small amount of alkali was added, and that once stained the micro-organisms were resistant to destaining by acid and alcohol. It is of interest to note that Hansen,<sup>2</sup> who first described "rods" within lepra cells, stained them with osmic acid, well known as a fat stain.

In 1882 Koch<sup>3</sup> demonstrated the etiological agent of tuberculosis in tissues, staining thin sections with a faintly alkaline aqueous solution of methylene blue, and destaining and counterstaining at the same time with Bismarck brown. The tubercle bacillus stood out as a blue rod in a brown field. Koch recorded in his original article that of all the bacteria he had examined only the leprosy bacillus behaved similarly. All others became brown in the Bismarck brown treatment.

Shortly afterwards Ehrlich<sup>4</sup> developed destaining methods for the establishment of acid-fastness, reporting that the tubercle bacillus once stained with basic fuchsin or other dyes in aniline water could not be decolorized by the use of 33 per cent nitric acid (one part concentrated nitric acid and two parts water) and alcohol. The modern Ziehl-Neelsen technique and other methods are essentially modifications of Ehrlich's<sup>5</sup> original procedure.

<sup>1</sup> Virchow's Archiv., 1881 (84), 514.

<sup>2</sup> Virchow's Archiv., 1880 (79), 32.

<sup>3</sup> Berl. klin. Woch., 1882 (19), 221.

<sup>4</sup> Deut. med. Woch., 1882 (8), 269.

<sup>5</sup> Deut. med. Woch., 1882 (8), 451. Deut. med. Woch., 1883 (9), 247; Fortschr. der Med., 1885 (3), 200; footnote in article by Johne.

These two organisms held their unique position as the only known "acid-fast" organisms but a short time, however, for in 1884, Lustgarten made a mistaken announcement of the cause of syphilis, describing acid-fast bacilli in the neighborhood of specific urogenital lesions. That similar or identical organisms occurred normally around the external genitalia and elsewhere on moist skin, was shown in the following year by Alvarez and Tavel, and the place of the smegma bacillus as a harmless acid-fast saprophyte was soon confirmed by many other investigators.

Since 1884 about fifty different types of acid-fast bacilli have been described. The best known of these are the avian type tubercle bacillus, isolated by Nocard in 1885, the bovine type of tubercle bacillus, established as a type distinct from the human type bacillus by Theobald Smith in 1898; a series of acid-fast bacilli occurring in the tissues of cold blooded animals, including the carp bacillus of Dubard, the turtle bacillus of Friedmann and the frog bacillus of Rupprecht; a group found in butter and other dairy products, first described by Petri and extensively investigated by Rabinowitsch; a variety of avirulent acid-fast microorganisms found in pulmonary abscess and gangrene, by Fraenkel, Pappenheim, Rabinowitsch and others, which might be confused with tubercle bacilli; and a number of bacilli found on various plants and grasses, first described by Moeller.<sup>6</sup> It is now known that other microorganisms than the true bacteria may be acid-fast. Some of the actinomycetes are resistant to acid and alcohol destaining.<sup>7</sup>

Although as fast as these organisms have been discovered, they have automatically been filed with the other bacilli characterized by the property of acid-fastness, question has not unnaturally

<sup>6</sup> The reader is referred to Potet (*Étude sur les Bactéries dites "Acido-philes,"* les paratuberculibacilles, Paris, J. B. Baillière et fils, 1902) and to McFarland ("A Textbook upon the Pathogenic Bacteria," Joseph McFarland, Philadelphia, W. B. Saunders and Co., 1910) for the literature upon these organisms, and to a recent compilation by A. K. Krause, (*Jour. Outdoor Life*, 1920 (17), 222). Covie (*Jour. Exp. Med.*, 1900-01 (5), 208) may also be consulted, especially for the literature on the smegma bacillus.

<sup>7</sup> For literature see Henrici and Gardner, *Jour. Infect. Dis.*, 1921 (28), 232.

been raised as to the propriety of classifying them together simply on the basis of this single tinctorial peculiarity. A number of problems of more than immediate practical interest are concerned. It goes without saying that the distinction between true tubercle bacilli and the avirulent smegma and grass bacilli is a matter of the utmost clinical and sanitary importance. But the question strikes much deeper, the very fundamentals of evolution being involved. In no group of organisms, not excluding the colon-typhoid and streptococcus-pneumococcus groups, has the problem of transmutation been more debated. For not only are there those who claim that the human type bacilli may become bovine type and vice versa but there are not a few who believe that after a more or less prolonged residence in mammals the bacilli of poikilothermic animals may become pathogenic for the isothermal and vice versa. To others, bacilli of cold blooded animals are nothing more than grass bacilli maintaining existence in an animal body; and accordingly, if the others be right, grass bacilli after an appropriate residence in a cold blooded host and later transmission to man, might be transmuted into virulent tubercle bacilli! When we add that it has been reported that a variety of non-acid-fast bacteria have been made to become acid-resistant by growth on fat-rich media, the situation verges on absurdity. All bacteria could become tubercle bacilli.

For a consideration of the probability of transmutation the reader is referred to an investigation by Weber and Taute<sup>8</sup> and to a compilation of the views of these and other observers by A. K. Krause.<sup>9</sup> The opinion of these more conservative authors is that the different acid-fast bacilli are well fixed in type. It is admitted that a given organism may vary its habitat from the surface of a mass of moss or timothy grass to a site of lowered resistance in a cold blooded animal; but it will remain there a long period of time without changing its essentially saprophytic nature.

It should be added that at the present time not all investigators are inclined to agree with these views. Particularly a group including Kolle, Schlossberger, Pfannenstiel and Igersheimer,<sup>10</sup>

<sup>8</sup> *Tuberk. Arb. a.d. kaiserl. Gesundheitsamt*, 1905.

<sup>9</sup> *Jour. Outdoor Life*, 1920 (17), 260.

<sup>10</sup> *Deut. med. Woch.*, 1921 (47), 437; *ibid.*, 1921 (47), 526.

report that the bacilli of cold blooded animals can be rendered virulent for the guinea pig by repeated animal passage. Jaffe<sup>11</sup> goes even farther, stating that butter and timothy bacilli, avian type tubercle bacilli and turtle bacilli, can call forth in the mouse necrotic lesions very similar to those of tuberculosis, and that the likeness is increased with animal passage.

Lange<sup>12</sup> and others have not confirmed such results, finding that passage of saprophytic strains through animals does not promote virulence. Lange's results are suggestive with regard to the supposed positive findings of others. In the case of his animals, bacilli were recognizable at the site of inoculation and in adjacent organs for forty-two weeks and culturable for thirty-two weeks. The fact must not be lost sight of that the formation of a tubercle is largely a foreign body reaction elicited by a difficultly digestible material. The acid-fast saprophytes are characterized, as is the tubercle bacillus, by a digestion-resistant, fatty or waxy protoplasm. What is more natural than that they should stimulate the formation of a tubercle, even with anemic necrosis, and that as a result of phagocytosis and lymphatic transportation some should be carried to adjacent organs? This process, with a gradual dying out of the organisms, should be distinguished from *tuberculosis*, in which multiplication of organisms occurs.

While the subject of inoculation is being considered it should be recalled that an etiological relationship between the acid-fast organisms isolated from the nodules of lepers and the disease leprosy, has never been established by definitely positive results with animal inoculation. Whether some unknown, additional factor is concerned in leprosy, or whether, even, the acid-fast bacilli which have been isolated are not identical with those seen in the lepra cells, still remains to be settled. In view of the failure of animal inoculation experiments to settle such points, and in view of the conflicting results obtained on animal inoculation with other acid-fast organisms, it has seemed to the author of this chapter that experiments on the metabolism and chemical composition of the members of the acid-fast group might result in very practical and useful additions to our knowledge.

<sup>11</sup> Arb. a. d. Staatsinst. f. exp. Therap. v. d. Georg Speyer Hause z. Frankfurt, A. M., 1921 (14), 3.

<sup>12</sup> Deut. med. Woch., 1921 (19), 528.

Whatever be the ultimate outcome of more exact experiments concerning the relationship between the acid-fast bacilli, there is good reason for believing that such relationship is a much closer one than a mere accidental similarity in staining reaction. In the first place, as will be brought out below, they have a factor in common and differ from other bacteria in the possession of a large amount of lipin. Secondly, serological reactions indicate that their antigenic activity is qualitatively the same. This has been known for many years. Robert Koch noted that the serum of tuberculous patients, inactive toward diphtheria, typhoid and plague bacilli, agglutinated human and bovine type tubercle bacilli and a great variety of other acid-fast organisms, including fish, blindworm, and butter and grass bacilli. Straus<sup>13</sup> and others found that tuberculin could provoke a general reaction in both nodular and anesthetic leprosy.

The elaboration of this line of investigation has led to an enormous amount of research on the immunizing properties of the acid-fast saprophytes toward tuberculosis. On the whole the results are to be considered negative.<sup>14</sup>

J. V. Cooke<sup>15</sup> has compared the antigenic value of a number of acid-fast organisms in the complement fixation test and finds that in the sera of rabbits immunized with various acid-fast bacteria there is present a complement-fixing antibody which reacts with all members of the group. He therefore believes the term "acid-fast fixation of complement" appropriate. It has been repeatedly found that tuberculous patients or artificially infected animals will react to "tuberculins" prepared from a variety of the saprophytic acid-fasts.

Such results, according to the usual concepts of immunology, indicate the common possession of certain proteins not present in the bodies of other bacteria, antigens being generally considered exclusively protein in nature. There is one conspicuous exception to this view. Hans Much<sup>16</sup> believes that the lipins of the

<sup>13</sup> Semaine Méd., 1893 (13), 364.

<sup>14</sup> Löwenstein's section on Immunization in Kolle and Wassermann's Handbuch d. Path. Mikroörg. may be consulted for a consideration of this subject.

<sup>15</sup> Jour. Infect. Dis., 1919 (25), 452.

<sup>16</sup> See Much and Leschke, Beit. Klin. Tuberk., 1911 (20), 343.

tubercle bacillus are primarily concerned in its immunological reactions, and that different lipin fractions have different antigenic values, the sum of which is nearly equal to that of the whole bacillus, the protein fraction having a small antigenic value. Deilmann<sup>17</sup> has amplified the claims of Much, finding that the other acid-fast bacilli contain lipin antigens in common with the tubercle bacilli, although a quantitative distinction can be drawn between bacteria of the various types. Whether they be protein or lipin in nature, the possession of common antigens has been interpreted as supporting the hypothesis that all of the acid-fasts have a close phylogenetic relationship.

#### CHEMICAL COMPOSITION OF THE ACID-FAST BACTERIA OTHER THAN TUBERCLE BACILLI

Chemical investigation of acid-fast bacilli other than the tubercle bacillus, has not been very extensive. It has been more concerned with the leprosy bacillus than with the rest of the group, and is mostly micro-chemical in nature, based chiefly upon histological study of tissue preparations.<sup>18</sup> A careful micro-chemical study of the fatty substances was made by P. Unna, Jr., who found that treatment of leprosy bacilli by fat solvents rendered them swollen and full of vacuoles. From the vacuolization he concluded that the body of the bacillus contained fat-rich and fat-poor portions. He observed, too, that those staining reactions which depended upon the presence of free fatty acids were not obtained after treatment of the organisms with alcohol and ether, nor was there persistence of the property of blackening osmic acid, generally attributed to the presence of unsaturated fatty acids, free or combined. Inasmuch as the bacilli stained only weakly with sudan and scharlach, neutral fats were considered to be present in small amount only. It was noted that after extraction of the sections with fat solvents, these dyes failed to stain the

<sup>17</sup> Zeit. Immunität., 1911 (10), 421.

<sup>18</sup> Jadassohn's (Kolle u. Wassermann Handbuch d. Path. Mikroorg. 1913 (5), 805.) article on leprosy may be consulted for the literature upon this subject.

organisms at all. Cholesterol could not be recognized microchemically. After extraction with alcohol, ether, chloroform and benzene the bacilli were alcohol-fast but not acid-fast. The fats removed were acid but not alcohol fast. Unna, Sr. agreed with P. G. Unna, Jr., that acid-fastness in the leprosy bacillus was due to an intimate mixture of an acid-fast fatty body with an alcohol-fast ground-substance, probably protein in nature.

The resistance of the leprosy bacillus in sputum and nasal secretion to antiformin, which is generally assumed to be a function of its fatty envelope, has been commented on by several investigators, particularly by Uhlenhuth and Steffenhagen.<sup>19</sup> It is distinctly less than that displayed by tubercle bacilli. According to Uhlenhuth and Steffenhagen morphological integrity may be maintained after treatment with 5 to 10 per cent antiformin, but not with stronger solutions, while tubercle bacilli are resistant to 25 per cent. Whether leprosy bacilli so treated remain alive could not be determined.

With the isolation of acid-fast bacteria from leprous lesions by Clegg<sup>20</sup> and Duval<sup>21</sup> and introduction of methods for growing the organisms in quantity, it became possible to make accurate studies on the chemical composition of the bacilli thus isolated. The value of these, as respects leprosy, is somewhat questionable in view of the still doubtful relationship of the isolated organisms to the pathological process of leprosy. Gurd and Denis<sup>22</sup> determined that the organism isolated by Duval contained 63.4 to 78.8 per cent water, a much smaller proportion than that usually found in the tubercle bacillus, which is about 90 per cent. The dried bacilli contained 36.4 per cent of material soluble in chloroform, of which 34.7 per cent was acetone-soluble and 1.7 per cent acetone-insoluble. The latter fraction, on repeated purification by acetone precipitation, was found to contain phosphorus and was considered on that basis to be lecithin. Blackening of the fat with osmic acid was taken as an indication of the presence of oleic acid. One and two-tenths grams of the crude lipin extract

<sup>19</sup> *Lepra*, 1909 (9), 94.

<sup>20</sup> *Philippine Jour. of Science*, 1909 (4), 403.

<sup>21</sup> *Jour. Exper. Med.*, 1910 (12), 649.

<sup>22</sup> *Jour. Exper. Med.*, 1911 (14), 606.

on saponification with alcoholic potassium hydroxide yielded 0.001 gram of a substance giving the color tests for cholesterol. The yellow pigment of the organism was readily soluble in fat solvents and accordingly seemed to belong to the group of lipochromes. The residue after the chloroform treatment was considered to be chiefly nucleoprotein. It dissolved in dilute sodium hydroxide, gave the biuret, Molisch, xanthoproteic, Millon and Hopkins-Cole tests. The nitrogen content was 8.2 per cent. The presence of sulphur and phosphorus was demonstrated. On hydrolysis with hydrochloric acid, neutralization with ammonia and treatment with ammoniacal silver, silver compounds of the nuclein bases were precipitated. The glycogen-like body described by Levene as occurring in tubercle bacilli could not be found, nor could any soluble substance reducing Fehling's solution.

A crystalline lipin product from an acid-fast organism designated *Strep. leproides*, has been placed on the market by Deyke.<sup>23</sup> It is prepared by washing the organism in water, grinding in ammonia water, treating with Adams' solution, washing in water, drying *in vacuo*, extracting with carbon disulphide, benzene or chloroform, distilling off the solvent and crystallizing the residue from alcohol or olive oil. From olive oil the product crystallizes in the form of flat white needles, melting at 48 to 52°, soluble in ether, chloroform and boiling alcohol. It is insoluble in cold alcohol, contains 92 per cent fatty acids, has a saponification number of 210, takes up 14 to 15 grams iodine per 100 grams, and contains no volatile fatty acids. It is claimed to be a solvent for leprosy and tubercle bacilli and to possess immunizing properties.

Pfannenstiel<sup>24</sup> (who gives a comprehensive bibliography) has published analyses comparing the lipin content of tubercle bacilli of human and avian types, frog bacilli, turtle bacilli, timothy grass bacilli, butter bacilli and butter bacilli which had been raised in virulence by guinea pig passage. The total lipin removed in Pfannenstiel's extractions was low, the highest recorded value being 16.47 per cent of the dry weight, in the case of a butter bacillus. The lowness of their figure is to be attributed to the method used, extraction of the dried material, which had been grown on glycerol

<sup>23</sup> German patent assigned to Kolle and Co., U. S. 972, 345.

<sup>24</sup> Zeit. f. Hyg. u. Infek., 1922 (95), 87.

broth, with a mixture of ether and acetone. As is well known some of the waxy material of the tubercle bacillus is difficultly soluble in ether, and the phosphatids, which as Tamura<sup>25</sup> has shown make up a considerable proportion of tubercle bacillus lipin, and some of the wax are insoluble in acetone. However, Pfannenstiel stresses the point that the figure obtained varies with the method, and as in his experiments the same method was used throughout, the results are of value for comparison of the different bacteria examined.

His results indicated a wide variability in lipin content throughout the group, a value as low as 2.85 per cent of the dry weight being found for a tubercle bacillus of the human type. It was thought that the lipins were distributed in the cell protoplasm in varying fashion and that accordingly ease of extraction varied. The figures do not indicate any constant quantitative difference in the lipin content of virulent and avirulent strains. Qualitative examination of the extracted material was not made.

Long and Campbell<sup>26</sup> have reported a series of analyses of acid-fast organisms for total and non-saponifiable lipin. The method

#### Lipin content of acid-fast bacilli

|                                     | TOTAL LIPIN IN<br>PER CENT OF<br>DRY WEIGHT | SAPONIFICATION<br>NUMBER | PER CENT OF<br>TOTAL LIPINS<br>AS NONSAPONI-<br>FIABLE LIPOID |
|-------------------------------------|---|--------------------------|---|
| H 37 human type tubercle bacillus.  | 22.7  | 131                      | 77.1  |
| B 1 bovine type tubercle bacillus.. | 22.3  | 139                      | 60.0  |
| A 1 avian type tubercle bacillus... | 11.0  | 194                      | 35.7  |
| Leprosy bacillus (Duval).....       | 9.7   | 188                      | 27.2  |
| Turtle bacillus.....                | 36.3  | 191                      | 28.3  |
| Frog bacillus.....                  | 37.1  | 195                      | 33.7  |
| Smegma bacillus.....                | 35.6  | 128                      | 4.6   |
| Dung bacillus.....                  | 34.7  | 125                      | 5.4   |
| Grass bacillus.....                 | 23.4  | 147                      | 9.4   |
| Grass III bacillus.....             | 30.3  | 109                      | 4.5   |
| Timothy bacillus.....               | 20.2  |                          |   |

#### Lipin content of other microorganisms

|                                   |     |  |  |
|-----------------------------------|-----|--|--|
| <i>B. subtilis</i> .....          | 4.4 |  |  |
| <i>Staphylococcus albus</i> ..... | 2.8 |  |  |

<sup>25</sup> Zeit. physiol. Chem., 1913 (87), 85.

<sup>26</sup> Amer. Review Tuberc., 1922 (6), 636.

of extraction has been given on p. 16. Their results are given on page 113. With two exceptions they indicate a very high lipin content for acid-fast as compared with non-acid-fast bacilli. A staphylococcus and a hay bacillus analyzed by the same method showed only 2.8 per cent and 4.4 per cent of the dry weight as lipin. In the case of these two exceptions, the avian bacillus and the leprosy bacillus, it was noted that a large amount of lipoid material, perhaps 20 per cent of the dry weight, which was soluble in warm alcohol, but insoluble in petroleum ether, was present. This substance requires further investigation. The other bacilli ranged from 20 per cent to nearly 40 per cent lipin.

A more significant finding however is that of the high wax content of the virulent forms. At one end of the scale we have organisms of scarcely questioned saprophytism, the smegma and grass bacilli, ranging in wax content (non-saponifiable lipin) from 4.5 per cent to 9.4 per cent. At the other end are the tubercle bacilli of human and bovine type with wax values of 77 per cent and 60 per cent of the total lipin. In between are organisms of debated pathogenicity, avian, leprosy, frog and turtle bacilli, with values for wax of 27.2 per cent to 35.7 per cent. The saponification numbers agree with the wax findings except in the case of the smegma and grass bacilli, where they are unexplainably low. Further research is necessary on this point. It is to be believed that the low wax content of these bacilli means a correspondingly high content of neutral fat, but the saponification number does not bear this supposition out. An excellent agreement with theory as respects saponification number and wax content is obtained in the case of the tubercle bacilli and the avian, leprosy, frog and turtle bacilli. We have every reason for believing that the greater proportion of the extracted lipin in the four last named types was true fat.

The authors would not lay too much stress upon their findings with respect to virulence, however. The virulence of most of the particular strains used has not been tested recently in their laboratory. It is known that H 37 and B 1 are highly virulent, but no attempt has been made to study virulence in the others, in any

animals. However the nutritional requirements of this group have been investigated<sup>27</sup> and are well understood. As is generally known, glycerol is an important element in the nutrition of the tubercle bacillus. The bacteria used in this investigation decrease in their glycerophilism approximately in the order in which they are listed in the table on page 113.

It is my belief, on the basis of these results and Frouin's, that the glycerol requirement and the wax content are to be directly correlated, glycerol being a wax-progenitor but not necessarily a fat-progenitor.

#### METABOLISM OF THE OTHER ACID-FAST BACTERIA

It is only in recent years that much attention has been paid directly to the nutrition of the avirulent acid-fast organisms.<sup>28</sup> However, the first isolation of an acid-fast bacillus from a leprosy nodule was dependent upon a nutritional peculiarity of the organism. Clegg<sup>29</sup> found that while there was no development of acid-fast bacilli when bits of leprous tissue were transplanted to the usual bacteriological media, growth would occur if the transplantation was made to cultures of amoebae or *Sp. cholerae* on the same media, that is, that the acid-fast organism was able to proliferate on isolation only in a condition of symbiosis. Mixed cultures of this sort were carried along for three or four "generations" with weekly transplantings, and then heated to 60° for thirty minutes. This temperature killed all organisms present except the leprosy bacillus, which was thus obtained in pure culture. After this it was able to multiply on practically all of the common bacteriological media under aerobic conditions. It did not produce gas nor form acids in dextrose and lactose media.

Duval<sup>29</sup> repeated Clegg's work, verifying it, but discovering that certain modifications of the medium could be used instead of the condition of symbiosis. He used a variety of bacterial symbiotics

<sup>27</sup> Long, Amer. Review Tuberc., 1922, (5), 857.

<sup>28</sup> For a consideration of bacterial nutrition in general the reader is referred to an article by Kendall (Jour. Infect. Dis., 1922 (30), 211) and to a series of articles by Rettger and his associates. (Jour. Biol. Chem., 1915 (20), 445; Jour. Bact., 1916 (1), 15.)

<sup>29</sup> Jour. Exp. Med., 1910 (12), 649; 1911 (13), 365.

successfully, and came to the conclusion that they promoted the growth of the leprosy bacillus in the medium by the production of protein disintegration products, which the leprosy bacillus, unaided, was unable to form from the protein molecule. If amino acids were used, particularly tryptophane, it was unnecessary to use other bacteria. Furthermore, protein media, such as coagulated egg albumen or blood serum, could be used if a few cubic centimeters of 1 per cent trypsin solution were added aseptically from time to time. Like Clegg he found that after a few weeks' growth on such media the bacillus developed the power of maintaining independent existence on the usual media.

Kendall, Day and Walker,<sup>30</sup> in their "Studies on Acid-fast Bacteria," found that a strain of smegma bacillus and one of grass bacillus split off ammonia from the protein constituents of peptone broth, although not so actively as the rapidly growing tubercle bacilli which they had previously studied. The leprosy bacillus of Duval produced less ammonia, and furthermore utilized ammonia to such an extent that after a few days' culture less ammonia was present than at the start. The grass and smegma bacilli on the other hand effected an increasing proteolysis as shown by the ammonia content of the medium, followed by a gradual recession of the ammonia, a phenomenon similar to that observed with tubercle bacilli. It was noteworthy that in cultures of these two organisms neither glycerol, dextrose or mannite exerted any appreciable sparing action for the protein constituents of the broth, ammonia production being practically the same in media containing these substances as in plain broth.

The same authors<sup>31</sup> reported that, like the tubercle bacillus group, smegma and grass bacilli formed lipase (esterase for ethyl butyrate and castor oil) during their growth in glycerol broth, which could be detected in the medium freed from bacteria. It was non-diffusible through either agar or a collodion membrane and resisted an exposure of fifteen minutes to 100°C. in the moist state without appreciable diminution in its activity. In a supplemental communication<sup>32</sup> it was pointed out that there was a

<sup>30</sup> Jour. Infect. Dis., 1914 (15), 439.

<sup>31</sup> Jour. Infect. Dis., 1914 (15), 443.

<sup>32</sup> Jour. Infect. Dis., 1914 (15), 467.

tendency for the period of maximum lipolytic activity of the broth to coincide with the period of maximum proteolysis. The Duval organism, which was relatively inactive as an ammonia producer, also produced little lipase. It was suggested, in view of this relation between maximum metabolic activity and lipolytic activity that the presence of the exolipase in the broth was not purely adventitious, but might play some rôle in the assimilation of food substances.

I<sup>27</sup> have made an attempt to group acid-fast organisms on the basis of nutrition. A number of these bacilli were cultivated upon simple media of known composition with the idea of testing their ability to utilize certain chemical arrangements of atoms. Nitrogen was furnished in well defined protein decomposition products, in amino, amide, amine or imino form. Carbon was supplied in the form of amino acids or certain alcohols and acids. Phosphorus, sulphur, potassium, sodium, calcium, magnesium and chlorine were kept constant in all experiments, the media containing 0.5 per cent sodium chloride,  $\frac{N}{50}$  potassium acid phosphate and traces of magnesium sulphate and calcium chloride. In addition, 2 per cent purified agar was used in all experiments except those with tubercle bacilli (human and bovine types). This was considered an inert substance. Cultures were run in quadruplicate.

Tubercle bacilli of human, bovine and avian type, leprosy bacilli, frog, fish and turtle bacilli, smegma bacilli and various strains of grass bacillus were used. In general it was noted that the NH<sub>2</sub> group of amino acids was utilizable by all of these bacilli as their sole source of nitrogen. Certain amino acids were not very readily utilizable, however, because of the presence of a somewhat toxic ring structure in their make up. Tryptophane and phenylalanine, containing respectively the indol and benzene nuclei, were in this group. Histidine, however, containing the imid-azol nucleus, was generally utilizable. The carbon residue left after the deaminization of amino acids was useless to the true tubercle bacilli in the absence of glycerol, these organisms being strongly glycerophilic. For the other bacilli, the amino acids alanine and leucine were sufficient as sole sources of carbon as well as nitrogen. The leprosy bacillus of Duval, however, which was able to use histidine as a sole source of nitrogen in the presence of another source of

carbon, as glycerol, was unable to grow when histidine constituted the sole source of carbon. Frog, fish and turtle bacilli behaved in a similar manner. Apparently these bacilli were unable to split the imidazol ring. Smegma and grass bacilli were able to use histidine as sole source of both nitrogen and carbon.

The acid amide group was utilizable by all of the bacilli, as was ammonia. Utilization of the amino group of primary amines was confined to three organisms, a frog bacillus and two strains of grass bacillus. The amino nitrogen of creatinine was not used by tubercle bacilli of human, bovine or avian types, was apparently used to some extent by leprosy bacilli, and was readily utilized by the bacilli of cold blooded animals and smegma and grass bacilli.

The nutritional characteristics noted thus provided data for classifying groups among these bacteria. The fact that alcohols other than glycerol, and organic acids, were insufficient sources of carbon for the true tubercle bacilli, which needed something in addition, preferably glycerol, set these bacilli apart from the rest of the group. The complex imid-azol-propionic acid structure of histidine was utilized by smegma and grass bacilli as a source of carbon and not by the others. Approximately the same separation could be made on the basis of the utilization of the nitrogen of tryptophane. Utilization of creatinine imino nitrogen placed the smegma bacilli with the grass bacilli and both of them apart from the tubercle bacilli. By exclusion of these two extremes the leprosy bacilli, provisionally so called, and the bacilli of cold blooded animals were left. In their utilization of the carbonaceous portions of d-l alanine and leucine, these behave somewhat alike and more like the grass and smegma bacilli than like the tubercle bacilli. In their less general utilization of propionic and lactic acid and ethyl alcohol as sources of carbon, they act more like the tubercle bacilli. In their action on creatinin they are again more like the grass bacilli. In their failure to use the carbonaceous part of histidine they behave similarly and resemble more the tubercle bacilli.

From the above considerations it will at least be seen that the tubercle bacilli on the one hand and the smegma and grass bacilli on the other, fall rather readily into separate nutritional groups.

The leprosy bacillus of Duval and cold blooded animal bacilli have many points of difference from both of these and certainly a few nutritional factors in common. Tentatively they can perhaps be considered together as a third group.

#### SUMMARY ON ACID-FAST BACILLI

Forty or fifty different strains of acid-fast bacilli have been isolated, their individuality in bacteriological classification depending chiefly upon virulence and habitat in nature. Many of the acid-fast saprophytes described as distinct strains, as for example the grass bacilli of a given locality and the dung bacilli, isolated from cattle dung of the same region, are certainly to be considered identical. On the basis of their common staining peculiarity of resisting decolorization by acid and alcohol they have been classified as a special group in the usual bacteriological systems. There is evidence, moreover, that the acid-fastness is associated with a certain antigenic value in common, which can be demonstrated in a qualitative way, even if weight for weight in a given test the antigens are not of equal value. This factor indicates a similarity in chemical composition, in all probability the common possession of proteins specific for the group. That the lipins, in which the organisms are rich, may be concerned, however, is held by those who believe in the antigenic value of this class of compound. The common antigenic value has led to frequent attempts to use the saprophytic organisms as immunizing agents against tuberculosis. Definite success has not been achieved. Attempts have been made to transmute one variety into another, but the more conservative investigators believe this has never been achieved experimentally. The impression, however, remains, based upon the possession of acid-fastness and similar antigenic value, that a phylogenetic relationship does exist.

Chemical investigation has so far disclosed only one striking difference from other bacteria, a conspicuously high content of substances extractable with the usual fat solvents, which averages, for the group, at least 30 per cent of the dry weight of the organism. There are differences within the group in the type of lipin extracted. The tubercle bacillus differs from the saprophytes in the possession of a higher content of a waxy lipin.

On the metabolism side it has been learned that all members of the group, including the virulent forms, are able to thrive when ammonia, amino acids or acid amides are supplied as the sole source of nitrogen, splitting ammonia from the last two named types of compound. Certain of the saprophytes, however, seem to be the only forms able to hydrolyze the imino grouping or split off utilizable nitrogen from primary amines. The organisms of the group, while they are provided with proteolytic enzymes, are not protein liquefiers in the usual sense of the term, nor do they split off in appreciable quantity any of the well known protein aporrhegmatia, such as indole and the amines.

The virulent forms are less adaptable than the saprophytes in their choice of carbon, depending for active growth upon the presence of glycerol. Carbohydrates are utilizable but seem insufficient to replace glycerol for maximum growth of the virulent forms. A parallelism between virulence, glycerophilism and wax content has been drawn. The di, tetra, and other polyatomic alcohols do not seem utilizable by these bacilli. The saprophytes on the other hand make ready use of ethyl alcohol, and the three carbon atom organic acids or the carbonaceous portion of amino acids, as their sole source of carbon.

On the basis of nutritional characteristics the author has developed a tentative classification of the organisms of the group, the grounds for which are discussed in detail in the foregoing pages, in which the tubercle bacilli of human, bovine and avian type are placed in one group, smegma and grass bacilli in another, and the so called leprosy bacilli and tubercle bacilli of cold blooded animals in another, the third group having no other basis for classification together than a few nutritional characteristics in common and one or two decided differences from both of the other groups.

**SECTION II**  
**THE CHEMICAL CHANGES IN THE TUBER-**  
**CULOUS HOST**

BY

**H. GIDEON WELLS**



## CHAPTER V

### THE CHEMICAL CHANGES IN TUBERCULOUS TISSUES<sup>1</sup>

Tubercles are characterized by the presence of caseation and fibrosis in different proportions, with or without calcification, and hence the composition of different lesions will vary. Furthermore, in the lung, old tuberculous lesions are found to be the site of more or less dense incrustations with coal pigment and the dust particles that are also found deposited in the peri-bronchial glands, and varying in composition with the amount and character of the dust inhaled by the individual. As for the fibrous tissue elements, we have little knowledge of their chemical composition in any condition, and certainly not in tuberculosis. According to their histological appearance it is to be assumed that they are characterized by a low content in nucleoproteins, and consist chiefly of collagen. The only analysis of granulation tissue with which we are familiar, that of Hirsch,<sup>2</sup> corroborates this assumption. His material was from the castration tumors of swine, composed of an edematous mass of loose scar tissue with more or less active proliferating areas. This showed no striking chemical features, containing 18.12 per cent of solids, including 2.28 per cent of ether-soluble material, the proteins being low in sulphur, phosphorus and purines.

#### NECROSIS

Necrosis itself is accompanied by distinct chemical changes in the tissues, brought about by the autolytic enzymes of the necrotic cells themselves and by enzymes from invading leucocytes. While blood plasma permeating the necrotic area may bring in proteolytic enzymes also, this effect is usually overshadowed by the anti-enzymatic activity of the plasma. Necrosis

<sup>1</sup> This topic has been the subject of extensive study in this laboratory by George T. Caldwell, and parts of this section are taken from his review of the literature (*Jour. Infect. Dis.*, 1919 (24), 81).

<sup>2</sup> *Amer. Jour. Med. Sci.*, 1920 (159), 356.

in tuberculosis is at first a coagulation necrosis, followed by caseation. The coagulation seems to be partly that of the cell proteins themselves, and partly that of fibrinogen entering the necrotic area with the plasma. How the coagulation of cell proteins is brought about in tubercles and similar avascular necrotic tissues is not known; among the possibilities that have been suggested are acid coagulation by the action of acids formed in the asphyxiated cells, specific coagulating enzymes within the cells, and protein-coagulating products of the tubercle bacilli. Schmoll<sup>3</sup> has shown that necrosis occurring in tubercles is indeed associated with an almost complete coagulation of the cell proteins, but it is doubtful if this can be attributed to the protein-precipitating substances which Ruppel<sup>4</sup> described as among the products of tubercle bacilli.

The striking nuclear changes seen in necrotic areas are unquestionably the result of enzymatic action after the order of autolysis, for if the enzymes in a piece of tissue be destroyed by heating in boiling water this tissue will retain its structure and nuclear stain for months if implanted into the body of an animal, whereas an unheated piece of tissue loses its nuclear staining in a few days, the cytoplasm becomes granular, and it soon disintegrates completely.<sup>5</sup> Since the chromatin elements of cells are composed of nucleoproteins, the karyolysis and karyorrhexis that take place in tuberculous areas are presumably brought about by the enzymes which attack nucleoproteins.

*Karyolysis* and *karyorrhexis* are, then, the result of an autolytic process, which is perhaps due to intracellular proteases that act specifically on nucleoproteins, and which may be designated as *nucleases*. Nuclear staining by the usual methods is believed to depend upon an affinity of the acid nucleoproteins (in which the nucleic acid is not completely saturated by proteins) for basic dyes. Presumably in karyolysis the first step consists in a splitting of the nucleoprotein of the chromatin into nucleic acid and protein; this can be accomplished, according to Sachs, by the ordinary trypsin, and presumably, therefore, by the trypsin-

<sup>3</sup> Deut. Arch. klin. Med., 1904 (81), 163.

<sup>4</sup> Zeit. physiol. Chem., 1898 (26), 218.

<sup>5</sup> Wells, Jour. Med. Research, 1906 (15), 149.

like enzymes of the cells. Corresponding with this change we should expect the free nucleic acid to give an intense staining with basic stains, and this has frequently been described by those who have studied the cytological changes in anemic necrosis,<sup>6</sup> and called *pycnosis*. As supporting this view still further may be quoted Arnheim's<sup>7</sup> observation that in alkaline solutions the nucleus soon stains diffusely and weakly, and not at all after twelve to eighteen hours exposure; this is to be explained by the fact that nucleic acid is both dissolved and neutralized by alkaline solutions. Acids developed in injured cells may, by combining with the basic elements of the nucleoproteins, render them still more acid and highly basophilic; thus, in muscles showing waxy degeneration from accumulation of lactic acid the muscle nuclei will be found pycnotic. After the nucleic acid has been freed from the protein by the autolytic enzymes, it is still further decomposed by the "nuclease" or similar intracellular enzymes that have the property of splitting nucleic acid into the several radicals that compose it—corresponding with this change the hyperchromatic nucleus loses its affinity for stains and *karyolysis* is complete. When extensive necrosis occurs there will result, therefore, an increased elimination of purines, as was found by Jackson and Pearce<sup>8</sup> in animals with severe hepatic necrosis from hematotoxic serum.

A careful analytical study of the changes taking place in the autolyzing spleen, for the purpose of correlating the chemical and microscopical changes, has been made by Corper,<sup>9</sup> which corroborates the interpretation of necrosis advanced above. He found that during the stage when pycnosis is the chief feature there is no appreciable change in the nucleus; that is, the nucleic acid has not been split into free purines and the rest of its components; at this stage but little change has occurred in the lecithin, and a very slight amount of proteolysis is demonstrable. During the stage of karyorrhexis and karyolysis the most active

<sup>6</sup> Schmaus and Albrecht, Virchow's Arch., 1895 (138), supp., p. 1; Ergeb. allg. Pathol., 1896 (3), 486 (literature).

<sup>7</sup> Virchow's Arch., 1890 (120), 367.

<sup>8</sup> Jour. Exper. Med., 1907 (9), 569.

<sup>9</sup> Jour. Exper. Med., 1912 (15), 429.

disintegration is taking place, about one-fourth of the nucleic acid becoming disintegrated by the time all nuclear structures have disappeared; in the same period nearly half the lecithin (phosphatids) is hydrolyzed, while about one-fourth the coagulable protein has been hydrolyzed into non-coagulable compounds. After this stage the changes are very slow. It is somewhat surprising to find that when no vestige of nuclear substance remains in stainable form, there still remains three-fourths of the nucleic acid in an intact condition. Corper publishes a series of plates, together with the chemical details, thus establishing a standard whereby the histological changes can be interpreted in terms of the chemical changes which cause them.

#### CASEATION

This term is applied to a form of coagulation necrosis in which the dead tissue has an appearance quite similar to that of cheese. If we bear in mind the fact that cheese is a mixture of coagulated protein and finely divided fat, and that in caseation we have a coagulation of tissue proteins associated with the deposition of considerable quantities of fat, the reason for the gross resemblance of the product of this form of necrosis to cheese is apparent. Schmoll<sup>3</sup> has analyzed caseous material, and found it nearly free from soluble proteins or proteoses and this is confirmed by Jobling and Petersen.<sup>10</sup> The protein material is almost solely coagulated protein, which in its elementary composition is related to the simple proteins or to fibrin, and not at all to the nucleoproteins. The extremely small amount of phosphorus present in the caseous material indicates that the products of disintegration of the cell nuclei must diffuse out early in the process. Caseation is, therefore, characterized by a coagulation of the proteins and a dissolving out of the nuclear components. Schmoll does not explain the cause of the coagulation, however. It may be that it is the same as in the coagulation of anemic infarcts (since tuberculous areas are decidedly anemic), or possibly the tubercle bacillus produces substances coagulating proteins, as Ruppel states is the property of "tuberculosamin." Indeed, Auclair<sup>11</sup>

<sup>10</sup> Jour. Exp. Med., 1914 (19), 251.

<sup>11</sup> Arch. méd. expér., 1899, p. 363.

claims that the fatty substance that can be extracted from tubercle bacilli by chloroform is the cause of the caseation. Dead tubercle bacilli do not produce true caseation, however, according to Prudden and Hodenpyle and others, despite their ability to produce tubercles, hence the substance causing the necrosis evidently does not diffuse readily from the bodies of the bacilli.

Morse and Stott,<sup>12</sup> who review the literature on this topic, found that the oily material which can be extracted from dead tubercle bacilli with cold ether did not produce any lesions when injected into animals, but the waxy material which can be extracted with hot alcohol from the residue left after extracting the bacilli with ether, produces typical tubercles. These tubercles, however, like those produced by dead tubercle bacilli, show no caseation, which presumably is the result of products of metabolism of the tubercle bacilli. However, we have the statement of Auclair and of Camus and Pagniez that the fatty materials of the tubercle bacilli in the concentrated form of an extract do produce caseation, and Gaehlinger and Tilmont<sup>13</sup> report that the lipoids of liver extracts also produce caseation and formations resembling cold abscesses when injected subcutaneously, which these authors attribute to the presence in the liver lipoids of substances possessed of a considerable degree of acid-fastness. Recent studies in this laboratory have failed to corroborate the above statements that true caseation can be produced by lipoids, whether from bacteria or tissues.

Schmaus and Albrecht,<sup>14</sup> having studied caseation microscopically, state that the process consists of the death of the cellular elements and the appearance of a firm intercellular substance arising as a transudate, together with the precipitation of a fibrinoid material, probably not identical with fibrin but giving the typical microchemical reactions of fibrin. The formation of the caseous detritus follows, by a progressive breaking up of the fibrinoid material. To these substances are attributed the chief importance in the formation of the firm dry condition of

<sup>12</sup> Jour. Lab. Clin. Med., 1916 (2), 159.

<sup>13</sup> Compt. Rend. Soc. Biol., 1911 (71), 345.

<sup>14</sup> Virchow's Arch., 1896 (144 suppl.), 72.

the caseous mass. Along with the production of the intercellular substance goes the disappearance of the chromatin of the cell nuclei.

Caseous areas persist for extremely long periods of time without undergoing absorption, which indicates that the autolytic enzymes are destroyed early in the process, presumably by the toxins of the tubercle bacillus, since a simple anemic necrosis is followed by much more rapid autolysis. Corresponding to this, Schmoll found the chemical evidence of autolysis very slight indeed in caseous areas, and even when the caseous material breaks down to form a "cold abscess" the fluid differs from true pus in containing less free amino-acids, e.g., tyrosine is missing.<sup>15</sup> Caldwell also obtained lower figures for extractives in caseous than in normal tissues. Because of a lack of chemotactic substances no leucocytes enter to remove the dead material, in consequence of which caseous material gives no evidence of containing proteolytic enzymes, according to the Müller-Jochmann plate method. That the failure of absorption is not due to modification of the proteins into an indigestible form is shown by the rapid softening of caseous areas when, through mixed infection, chemotactic substances are once developed and leucocytes enter. Jobling and Petersen<sup>16</sup> suggest that in caseation the autolysis is inhibited by the soaps of fatty acids, which are abundant in caseous areas and have a marked antitryptic effect. Pesci<sup>17</sup> has reported that tuberculin somewhat reduces the rate of autolysis in vitro for a short time and then causes it to increase, but in such work as this the slight quantitative differences observed are of no significance, especially when the H-ion concentration of the autolyzing mixture is not known.

#### THE LIPINS OF TUBERCLES

Using fat stains, Rosenthal<sup>18</sup> found no evidence of fat in miliary tubercles unless there was some caseation present. The fat seems to make its appearance with the occurrence of the casea-

<sup>15</sup> See Müller, Cent. inn. Med., 1907 (28), 297.

<sup>16</sup> Jour. Exp. Med., 1914 (19), 239; Zeit. Immunität., 1914 (23), 71.

<sup>17</sup> Cent. f. Path., 1911 (59), 71 and 186.

<sup>18</sup> Verhandl. d. deutsch. path. Gesellsch., 1899 (2), 440.

tion. Within an extensive caseous area no fat was found, or at most only traces, while just at the boundary of the caseous area, fat-bearing cells were seen. Some of the giant cells in this region are said to resemble fat cells, while other giant cells were apparently fat-free. Streaks containing small fat droplets were sometimes seen in sections cut through fresh caseous areas. These are explained as representing the boundaries of small caseous areas which have fused to form the larger area, in which case the fat had not disappeared as it seems to do with the gradual extension of the caseation in the tuberculous tissue. Likewise, Vallillo,<sup>19</sup> in studying avian tuberculosis, observed that non-necrotic tubercles composed of epithelioid and giant cells contain either no stainable fat or only sparsely grouped droplets in the center. In the tubercles which had necrotic centers, the fat droplets were abundant and were located chiefly in the cytoplasm of the giant cells accumulated there. The fat droplets, however, were not numerous in the caseous part of the tubercles, as is also emphasized by Sata.<sup>20</sup> Similar observations were made by Hagemeister<sup>21</sup> and Herxheimer.<sup>22</sup> Fischler and Gross<sup>23</sup> could not demonstrate by microchemical methods the presence of free fatty acids in caseous areas. Chaussé,<sup>24</sup> using sudan III and osmic acid, demonstrated fat droplets in the giant cells and in practically all other cells in the tuberculous area and also in the substance derived from the destruction of all these cells. Joest<sup>25</sup> obtained results quite similar to those previously reported by Rosenthal. He was never able to demonstrate fat in the inter-cellular substances. Emphasis is placed on the observation that, although fat is demonstrable in the caseous part of the tubercle, it is less prominent there than in the boundary zone of the living tissue, and the living tubercle tissue apart from the boundary zone is free from stainable fat. It is explained, however, that this

<sup>19</sup> *La Tuberculosis*, 1911-12 (4), 257.

<sup>20</sup> Ziegler's *Beitr.*, 1900 (28), 461.

<sup>21</sup> *Virchow's Arch.*, 1903 (172), 72.

<sup>22</sup> *Ergeb. d. allg. Path. u. path. Anat.*, 1902 (8), 669.

<sup>23</sup> Ziegler's *Beitr.*, 1905 (7th suppl.), 344.

<sup>24</sup> *Compt. Rend. Soc. Biol.*, 1909 (64), 377.

<sup>25</sup> *Virchow's Arch.*, 1911 (203), 451.

impression of a smaller fat content in the caseous portion is undoubtedly due in part to the fact that the fat in the living cells of the boundary zone occurs in sharply circumscribed globules, while in the caseous area the globules are broken up and the fat more diffusely scattered. However, the peripheral distribution of the fat may indicate that it is mostly derived by migration from outside the tubercle.

In the study of pathological calcification, Wells<sup>26</sup> used both staining and chemical methods for the recognition of fats in tuberculous tissues. Specimens of human and bovine tuberculous lymph glands, stained with Sudan III, revealed marked infiltration with fine and coarse fat granules all through the areas that are acellular, the largest and most abundant granules being usually at the periphery. When counterstained with hematoxylin, the calcium deposits were found to lie in such tissues as were stained for fat, but there was no particular difference to be noted in the amount or character of the fat in the vicinity of the calcium deposits and elsewhere. Not infrequently a calcium deposit was noted at the periphery of the gland or tubercle, while, in the center, there was no calcium but many fat granules, although not more than in the tissues surrounding the calcium deposits.

Among the fatty materials at the margins of caseous areas are "myelin" drops, which consist chiefly of cholesterol esters.<sup>27</sup> In tuberculous lungs Babes<sup>28</sup> found areas rich in phosphatids and cerobrosides according to micro-chemical evidence, while in the caseous areas he found cholesterol esters, fatty acids and calcium soaps. He believes that the neutral fats appear only when they are formed by invading mononuclear cells.

Corper<sup>29</sup> used intravital staining methods in a study of the fat in the tubercles of guinea pigs. It had been shown by the work of Riddle and others that fat dyes such as Sudan III and scarlet R, entering the body dissolved in fat, remain either entirely or chiefly with this same food fat, being deposited with it if the

<sup>26</sup> Jour. Med. Research, 1906 (14), 491.

<sup>27</sup> Dietrich, Verh. deut. Path. Gesell., 1910 (14), 263.

<sup>28</sup> Compt. Rend. Soc. Biol., 1912 (72), 891.

<sup>29</sup> Jour. Infect. Dis., 1912 (11), 373.

food fat is deposited, but not leaving the food fat to enter either stored fat or the intracellular fats or lipoids of active tissues. Corper observed that the fats of tubercles in animals fed stained fats never contained any demonstrable amount of the fat dyes administered, no matter whether the tubercles formed before or after the animal was saturated with the dye. He states, therefore, that it seems probable that the fats microscopically visible or chemically demonstrable in tubercles, are derived chiefly or solely from the existing fats and lipoids of the disintegrated cells and are not deposited from the fats in the blood. This view is entirely in harmony with the histologic evidence. Furthermore, in support of this view of the origin of the fat in tubercles, we have the observation of Dietrich<sup>30</sup> that in simple anemic necrosis of the kidney the fat disappears less rapidly than the water and the other solids, so that we have produced a relative fatty increase without a corresponding absolute increase.

Bossart<sup>31</sup> analyzed materials of human origin; only 1 consisted of completely caseous material, the remaining 4 specimens ranged from one-sixth to one-third caseous substance obtained from lymph glands. The reported fat content in percentage of dry weight varied from 13.77 in a specimen which was estimated as one-sixth caseous, to 23.79 in a specimen one-fourth caseous. The total fat content of the pure caseous material is given as 20.75 per cent of the dry weight. The figures reported for the lecithin content are apparently of no great value since no lecithin at all was obtained in 3 out of the 5 specimens. In 3 specimens varying from one-sixth to one-third caseous, cholesterol ranged from 25.8 to 33.5 per cent of the total fats, while the latter are reported as varying from 13.77 to 15.73 per cent of the dry weight. In the completely caseous specimen, the cholesterol value is given as 2.77 per cent of the alcohol extract, apparently a much smaller amount of cholesterol than that found in the partially caseous specimens. The negative findings for lecithin reported by Bossart have not been substantiated by other workers with caseous material. Schmoll<sup>3</sup> was able to demonstrate con-

<sup>30</sup> Verh. Deut. Path. Gesell., 1907 (11), 15.

<sup>31</sup> Zur Chemie der Verfettung, Dissertation, Basel, 1902. Quoted by E. Schmoll, Deutsch. Arch. f. klin. Med., 1904 (81), 163.

siderable amounts of glycerol-phosphoric acid in the alcoholic extracts of all specimens of pure caseous material which he examined. He used 3 specimens of completely caseous material from bovine lymph glands, and 1 specimen of human material which was about one-fourth caseous. In connection with F. Müller, he analyzed the lipin fraction of a specimen of caseous material from human lymph glands. Cholesterol was found present but the amount is not stated. The phosphorus content of the ether-soluble material was 1.57 per cent, or when calculated as lecithin, 38.31 per cent. This corresponds to 3.83 per cent of the dried caseous material.

It has been suggested that the lipins found in tubercles may come in part from the bacilli themselves, but it is highly doubtful that bacillary lipins can constitute any appreciable proportion of the tubercle lipins. Certainly not all of the tubercle lipins can come from this source since cholesterol is a conspicuous feature whereas this substance is lacking in the tubercle bacillus.

#### GLYCOGEN IN TUBERCLES

In general, glycogen is found in quantities and conditions demonstrable microchemically especially in acute inflammatory conditions where there are numerous leucocytes, although it is also found in the fixed tissue cells. Chronic inflammatory processes usually exhibit very little visible glycogen, and this is true of tubercles.<sup>32</sup> Usually little or none is present, yet it has been found in both the leucocytes and epithelioid cells of tubercles, and occasionally even in the giant cells. In guinea pig tuberculosis it is more abundant, presumably because of the more rapid course, and Gierke found much glycogen visible in the epithelioid cells and in the adjacent connective tissue. Lubarsch states that it is most abundant in experimental tubercles sixteen to twenty days old, disappearing later. Devaux<sup>33</sup> found glycogen often in the same cells that contained tubercle bacilli. It was never found in the lymphocytes. The largest amounts were in the tissues immediately outside the zone of necrosis, but all cells showing nuclear destruction are free from glycogen. The glyco-

<sup>32</sup> See Gierke, Ergeb. allg. Pathol., 1907 (XI), 871.

<sup>33</sup> Ziegler's Beitr., 1907 (41), 598.

gen is, therefore, found in much the same places as the fat deposits. Very young tubercles, that as yet show no necrosis, are glycogen free.

The only chemical investigation that we can find is that of Huppert<sup>34</sup> who demonstrated the presence of glycogen in the contents of tuberculous abscesses, but in much smaller amount than in acute abscesses. Thus, in an acute pelvic abscess he found from 123 to 167 mgm. glycogen per 100 cc.; in acute phlegmons, 77.6 and 56.8 mgm.; in acute osteomyelitis, 72.6 mgm.; in a four months old psoas abscess pus, 2.8 mgm.; in a three months old tuberculous rib abscess, 0.41 mgm.; in a ten weeks old coxitis, traces only; pus from an old psoas abscess at the first emptying contained only 0.12 mgm. of glycogen per 100 cc., and when opened again twenty-three days later no glycogen at all could be found. It will be noted that the amount of glycogen varies in proportion to the number of leucocytes present in the pus; nevertheless, lymphoid tissues contain some glycogen (0.092 per cent, Lilienfeld) and hence there is presumably some in free lymphocytes.

#### THE ENZYMES OF TUBERCULOUS TISSUES

The various changes that take place in the tubercle are undoubtedly brought about for the most part by enzymes, but as yet little investigation has been made of the character of enzymatic processes in tuberculous as compared with normal tissues. This undoubtedly depends on the fact that we have as yet but inadequate and not too reliable methods for the quantitative study of the activity of tissue enzymes.<sup>35</sup> As stated previously, we recognize that autolysis must be slow in caseous areas, since they do not usually undergo liquefaction and persist for so long a time as solid masses of dead tissue that calcification commonly sets in. Since areas of simple anemic necrosis, i.e., aseptic infarcts, show much more complete and rapid absorption than necrotic tubercles, it seems probable that the tubercle bacilli must inhibit or destroy the autolytic enzymes, but, if so, the rate of action is

<sup>34</sup> *Zeit. physiol. Chem.*, 1894 (18), 143.

<sup>35</sup> See review on Intracellular Enzymes and Autolysis in Wells, *Chemical Pathology*, 4th ed. Chap. III.

probably too slow to permit of this fact being established by experimental methods.

Jobling and Petersen<sup>36</sup> found an emulsion of completely caseated material from lymph glands to show no autolysis whatever, nor could any evidence be obtained that active but inhibited proteolytic ferments were present. Material from caseous pneumonia exhibited somewhat more autolysis, especially in acid medium, since the caseous material could not be so completely separated from non-necrotic elements. They attribute the suppression of autolysis in tubercles to the presence in them of compounds of unsaturated fatty acids, mostly derived from the tubercle bacilli, which have a strong antitryptic effect somewhat in proportion to their degree of unsaturation. As establishing the importance of the unsaturation of the fatty acids, they cite the fact that when iodin is added to the inhibiting lipoids their antienzymatic effect is suppressed, which is attributed to a binding of the unsaturated bonds. They also ascribe the antitryptic effect of normal serum to its unsaturated lipoids.

As the nuclei undergo rather rapid changes in the early stages of tuberculous necrosis, we may assume that the *nucleases* which disintegrate the nucleoproteins that constitute the chromatin are present and active, at least at first. Caldwell's figures also give chemical evidence of the disintegration of the nucleoproteins, and Griniew<sup>37</sup> found experimentally an increase in the nuclease activity as shown by purine formation during autolysis of the tuberculous lung of guinea pigs, but this probably resulted merely from an increase in the number of cells present in the lung. After the first nuclear disintegration of the tubercle, however, the autolysis seems to be greatly delayed if not inhibited. That this effect is not due to changes in the dead proteins which render them indigestible is shown by the fact that when leucocytes are attracted into a tuberculous focus by secondary infection, or by injection of a chemotactic substance such as iodoform, digestive softening goes on rapidly. Pus from a cold tuberculous abscess will not digest fibrin, but if it becomes filled with leucocytes

<sup>36</sup> Jour. Exp. Med., 1914 (19), 251; Zeit. f. Tuberk., 1914 (22), 521; Jour. Lab. Clin. Med., 1915 (1), 172.

<sup>37</sup> Arch. Sci. Biol. St. Petersburg, 1912 (17), 176.

after injecting iodoform it will then do so (Heile).<sup>38</sup> Likewise, caseous material does not undergo appreciable autolysis unless it is infiltrated with leucocytes (Jobling and Petersen). Caseous pneumonia affords a striking comparison to croupous pneumonia in the slow rate at which it undergoes autolysis (Fr. Müller).

On the other hand, some evidence of proteolysis in tubercles has been reported. Matthes has obtained reactions indicating the presence of deutero-albumose and peptone in tuberculous glands which have not undergone complete caseation. Speithhoff,<sup>39</sup> continuing the studies of Matthes, found albumose and peptone, even though in very small amount, in all of 10 extracts prepared from the non-caseous, mixed with the caseous, parts of tuberculous lymphatic glands of cattle, and suggested that their presence may be referable to autolysis. In 7 of 10 instances in which caseous material was carefully freed from non-caseous tissue, these substances were not found, whereas in the remaining 3 specimens incomplete evidence of their occurrence was obtained. Although Caldwell found the amount of water-soluble material in caseous tubercles to be less than in the normal tissues, of this a much larger proportion of the nitrogen was in the form of proteose (11.65 to 35.2 per cent) than in the extract from normal tissues (7.81 to 9.37 per cent). The proportion of free amino acid nitrogen was about the same in both. Evidently, then, but little chemical evidence of autolysis can be found in tubercles, which corresponds with the morphological evidence of slow and incomplete autolysis.

As to the direct evidence concerning the enzymes in tuberculous lesions, Edw. Müller and Jochmann<sup>40</sup> found that pus-like fluid from a cold abscess, unlike pus of acute inflammation, is incapable of dissolving Löffler's blood serum when incubated at from 55 to 60°C., and Kolaczek and Edw. Müller<sup>41</sup> claimed that failure of a drop of pus-like fluid to cause a depression upon the surface of a plate of Löffler's serum may be used for diagnosis of uncomplicated tuberculous lesions.

<sup>38</sup> Zeit. klin. Med., 1904 (55), 508.

<sup>39</sup> Zent. inn. Med., 1904 (25), 481.

<sup>40</sup> Münch. med. Woch., 1906 (53), 1393.

<sup>41</sup> Ibid., 1907 (54), 253.

Opie<sup>42</sup> has found that those exudates and tissues which contain in abundance polynuclear leucocytes with fine granulation contain an enzyme which is distinguishable by its ability to digest protein in either an alkaline or a neutral medium. On the other hand, the large mononuclear phagocyte, which is often conspicuous in tuberculous reactions, contains an active enzyme which is distinguishable from that of the polynuclear leucocyte by its ability to digest protein in the presence of a weakly acid reaction. He has also demonstrated that epithelioid cells, which form the chief element of tuberculous tissue, contain an enzyme which causes active digestion of protein in an approximately neutral or in a weakly acid medium, but is inactive in the presence of weak alkali.<sup>43</sup> This enzyme resembles the enzyme which occurs in the large mononuclear cells of an inflammatory exudate and is more active than the similar enzymes of parenchymatous organs such as the liver. The enzyme which digests in the presence of acid exhibits greatest activity at a time when caseation is beginning. With advance of caseation its activity diminishes so that tissue which has undergone almost complete caseation exhibits little evidence of the presence of this enzyme. It is probable that complete caseation is followed by total disappearance of enzymes, thus accounting for the cessation of autolysis.

Tuberculous tissue contains an enzyme capable of digesting protein in the presence of alkali (leucoprotease) only during the early stages of its development. This enzyme, present at a time when the tissue contains numerous polynuclear leucocytes, quickly disappears so that when the enzyme digesting in acid is still active, leucoprotease has disappeared.

The serum of a tuberculous pleural exudate obtained by intrapleural inoculation with tubercle bacilli, causes slight inhibition of the mixture of enzymes contained in tuberculous tissue shortly after inoculation. The serum of blood causes complete inhibition of the enzymes contained in the same tuberculous tissue. Analysis of this difference indicates that the exuded tuberculous serum, like the serum of the blood, inhibits proteolysis caused by leucoprotease, but fails to inhibit digestion caused by

<sup>42</sup> Jour. Exp. Med., 1905 (7), 316.

<sup>43</sup> Opie and Barker, Jour. Exp. Med., 1908 (10), 645.

the enzyme acting in the presence of acid. The foregoing facts offer suggestions which may serve to explain in part the nature of the tubercle and the changes which occur within it. The so-called epithelioid cells of the tubercle resemble the large mononuclear phagocytes of inflammatory exudates and both contain an enzyme of the same character acting best in a weakly acid medium. It is not improbable that caseation which, like autolysis, is accompanied by disappearance of nuclei, is in part dependent upon the presence in the cells of this active proteolytic enzyme. Injury to cells by products of the tubercle bacillus, or partial anemia the result of imperfect vascularization of the tuberculous tissue, may have a part in rendering these cells susceptible to self-digestion. Changes which have been observed in serum of the tuberculous exudate show that the anti-enzymatic property of the normal blood may be absent in the exudate of a tuberculous lesion. This loss of anti-enzymatic action, perhaps referable to changes caused by products of the tubercle bacillus, may favor self-digestion of the enzyme-containing cells and diffusion of their enzyme. Jobling and Petersen<sup>44</sup> ascribe the anti-tryptic action of normal serum to its content of lipoids with unsaturated fatty acids.

### *Lipases*

Since lymphocytes are rich in lipase, and according to Resch<sup>45</sup> are the source of the lipolytic enzymes of exudates, fat-splitting enzymes should be abundant in tuberculous lesions in the early stages with lymphoid infiltration, but we have found no investigations of this question. Pesci<sup>46</sup> found that tuberculin stimulates the hydrolysis of butyryl by tissue extracts, although not appreciably influencing the hydrolysis of oil or lecithin, but the correctness of this report has not been confirmed. Bergel<sup>47</sup> states that if drops of pus from tubercles are placed on plates of "yellow wax" (melting point 63 to 64°, origin not stated) and kept at 52°, small cavities are formed about the drops from local softening.

<sup>44</sup> Jour. Exp. Med., 1914 (19), 459.

<sup>45</sup> Deut. Arch. klin. Med., 1915 (118), 179.

<sup>46</sup> Pathologica, 1911 (3), 207.

<sup>47</sup> Münch. med. Woch., 1909 (56), 64; Berl. klin. Woch., 1921 (58), 995.

ing of the wax. Similar effects are produced by any material containing many lymphocytes (lymph gland and spleen tissue, exudates produced by tuberculin injection, blood in lymphatic leukemia). Hydrolysis of butter and oil may be produced by the same materials. On the other hand, Winternitz and Meloy<sup>48</sup> found that the capacity of the tuberculous lung to split ethyl butyrate was much lower than that of the normal lung, and the liver and kidney of tuberculous bodies show a decrease in butyrate activity, whether tuberculous or not. Nees<sup>49</sup> repeated Bergel's experiments and was not able to confirm them fully, especially in respect to the idea that fat splitting is a property more actively shown by lymphocytes than by other cells. In this laboratory we have been entirely unable to confirm Bergel's work.

Griniew<sup>37</sup> has determined the general enzymatic activity of dried and ground tissues from tuberculous guinea pigs, and he gives the following table of results:

| ORGANS AND TISSUES OF<br>TUBERCULOUS GUINEA<br>PIGS | PERCENTAGE DIFFERENCE OF THE PATHOLOGICAL CONTENT OF<br>ENZYME BASED ON THE NORMAL AS 100 |                       |  |                                |  |
|---|---|-----------------------|--|--------------------------------|--|
|   | H <sub>2</sub> O  | Lipase-<br>0.01 N KOH | Catalase-<br>O <sub>2</sub> from H <sub>2</sub> O <sub>2</sub> | Amylase-<br>starch<br>digested | Nuclease-<br>phosphoric<br>acid from<br>sodium<br>nucleate |
| Spleen.....   | +4.5  | -42.3                 | +11.4  | + 2.4                          | - 59.3   |
| Heart.....  | +3.3  | -39.5                 | +10.0  | -23.2                          | + 61.2   |
| Kidney.....   | +8.5  | -51.0                 | -17.7  | -11.0                          | - 20.0   |
| Brain.....  | +1.9  | -57.0                 | +41.1  | -29.8                          | + 0.6  |
| Lungs.....  | +4.3  | -55.6                 | -19.8  | -23.9                          | +114.5   |
| Liver.....  | +9.9  | -59.8                 | -13.2  | +12.9                          | - 18.7   |
| Muscles.....  | +7.7  | -50.5                 | +11.8  | +40.0                          | - 73.0   |
| Bone.....   | +6.1  | -60.0                 | -42.6  | + 2.5                          | - 82.7   |

It is, however, very doubtful whether quantitative results obtained in this way have any value whatever, especially in respect to indicating any alterations in function that may have existed in the living organism. Moreover, in this work, no consideration seems to have been given to whether the organs examined

<sup>48</sup> Jour. Med. Res., 1910 (22), 107.

<sup>49</sup> Biochem. Zeit., 1921 (124), 156.

were themselves tuberculous or not. The high nuclease activity in the lung, for example, might well depend on the increased number of nucleated cells in the infected lung, although no corresponding explanation could be found for the high figure with the heart.

Kotschneff<sup>50</sup> examined the organs of guinea pigs and rabbits that had received intraperitoneal injections of killed tubercle bacilli and reports that the lipolytic, amylolytic and catalytic activity were reduced, nuclease and antitrypsin increased. The significance of these observations is doubtful.

### *Oxidizing enzymes*

The so-called oxidase granules of cells, which give the indo-phenol blue test, are thought by some to represent true enzymes, possibly a phenolase, and hence may be considered here, although there is much doubt as to their enzymatic character. They seem to be present in nearly all cells if sought under proper conditions, but are most conspicuous in the neutrophile granules of leucocytes and myeloid cells of the bone marrow. Makino<sup>51</sup> finds that the less widespread, labile type of oxidase granule is present in the giant cells of tubercles, varying greatly in size, located mostly near the nuclear zone, and establishes thus the fact that the protoplasm of giant cells is an active, living substance, and not dead as Weigert maintained. He does not discuss the occurrence of these granules in the other elements of tubercles, but Gräff<sup>52</sup> states that the cells of tuberculous granulation tissue lack the oxidase granules. He also observed a decrease in the number of these granules in the tissues generally in persons dying of tuberculosis with cachexia. As we have no reliable methods for determining the oxidative activity of tissues the absence of data on tuberculous tissue is not to be regretted.

Also, since we do not know whether the reduction of methylene blue by the tissues depends upon the presence of a true enzyme, a reductase, it is not possible to estimate the significance of the

<sup>50</sup> Biochem. Zeit., 1913 (55), 481.

<sup>51</sup> Verh. Japan. Path. Gesellsch., 1915 (5), 71.

<sup>52</sup> Frankf. Zeit. Path., 1913 (12), 358.

finding by Günther<sup>53</sup> that the cellular elements of tubercles and the tissues immediately about them, show a decreased capacity to accomplish this reduction; furthermore this is in direct opposition to the statement of Pfeiffer<sup>54</sup> that lung tissue containing tuberculous lesions is especially active in reducing the dye.

Winternitz and Meloy<sup>55</sup> found a decreased catalase activity in the tuberculous lung, which they attribute to its small content of blood; a lowered activity observed in other organs is explained by the anemia and wasting. The existence of a possible catalase-inhibiting substance in tuberculosis was not investigated.

The feces in tuberculous enteritis show a high catalase activity, as in chronic colitis,<sup>56</sup> presumably from the increased content in tissue elements coming from the ulcers.

We can find no other reports of investigations on the distribution of oxidizing enzymes or related agents in tuberculous tissues, beyond a statement by Griniew<sup>57</sup> that in guinea pigs infected with tuberculosis the lungs, liver and kidneys show a subnormal catalase content, but nothing is said as to what extent these organs were the site of tuberculous lesions, if at all.

#### THE QUANTITATIVE COMPOSITION OF TUBERCULOUS TISSUES

Schmoll<sup>3</sup> extracted with cold water the residues of caseous *human* lymph glands freed from lipins, and found that no protein materials seemed to go into solution, as all the protein reactions were either completely negative or scarcely evident. Elementary analyses were made on the residue insoluble in alcohol, ether and water. The calculations made on the basis of the ash-free material gave the following averages for the three specimens of completely caseous material: carbon, 53.92 per cent; hydrogen, 7.38 per cent; nitrogen, 16.44 per cent, and sulphur, 0.65 per cent. The ash content varied from 9.2 to 23.3 per cent. Phosphorus was determined only on the specimen of caseous material containing 23.3 per cent ash and in this case it constituted 1.04 per

<sup>53</sup> Arb. Inst. Path. Anat. Tübingen, 1914 (9), 316.

<sup>54</sup> Beitr. Klin. Tuberk., 1921 (47), 46.

<sup>55</sup> Jour. Exp. Med., 1908 (10), 759.

<sup>56</sup> Norgaard, Jour. Biol. Chem., 1919 (38), 501.

cent of the ash-free substance. The ash value for the human tuberculous specimen, one-fourth caseous, was 4.63 per cent, with a phosphorus content of only 0.25 per cent. This exceptionally low percentage of phosphorus is stated as being surprising, since it was obtained from tuberculous but not completely caseated lymph glands. As this tissue is normally rich in phosphorus, this finding seemed to indicate that the process of coagulation necrosis, as appears evident also microscopically, is accompanied by a destruction of the cell nuclei and a washing away of the products formed. Whatever the sulphur content means, it was thought to be distinctly lower here than in most proteins. However, the value given is slightly higher than that given by Corper for the sulphur content of the dog spleen. In order to find out more concerning the character of the protein in caseous material, Schmoll studied its conduct toward pepsin-HCl digestion and hydrolytic cleavage with HCl. The fluid obtained by about two months digestion with the pepsin-HCl mixture showed that this protein substance differs in no essential way from other proteins so far as its conduct toward digestion is concerned. From the results obtained, Schmoll thought that he could exclude with certainty the existence of any nuclein material whatever, since no precipitate was obtained with ammoniacal silver solution and he remarks that the undissolved portion was certainly not nuclear material. Following hydrolysis with HCl, a partition of the nitrogen gave the following values:

|                                 |                          |
|---------------------------------|--------------------------|
| Humin and ammonia nitrogen..... | 5.01 per cent of total N |
| Basic nitrogen.....             | 43.9 per cent            |
| Amino-acid nitrogen.....        | 51.1 per cent            |

The low percentage of humin and ammonia nitrogen as well as the richness in basic nitrogen is noted as being remarkable. An attempt to determine guanine and adenine was unsuccessful. Schmoll studied also the autolysis of tuberculous caseous material and found that the autolytic processes go on extremely slowly in such tissues. He remarks that this may explain the fact that caseous material is so rarely absorbed.

Both human peribronchial glands and tuberculous mediastinal lymph glands of cattle have been studied by Wells<sup>26</sup> who called

attention to the fact that bovine tuberculous lesions differ from human lesions in that calcification occurs during the progress of the disease and is extensive in the form of innumerable sand like granules, scattered all through the tuberculous tissue even while the disease is in active stages. Calcification is usually an evidence of latency or healing in human tuberculous areas, and the deposits are found in much larger masses, each of which usually corresponds to an entire tubercle. Two sorts of bovine material were collected and examined separately in the course of this investigation. One consisted of the fluid, pus-like content of the large softened glands. This material escapes when the glands are opened and contains but few granules of calcium large enough to be felt by the finger. The other specimen was obtained by scraping the surface of unsoftened tubercles and the walls of the tubercle cavities. It consisted largely of the calcified material and the adherent tissue, mixed with more or less of the tissue elements but giving a fair conception of the substances immediately about the calcified masses.

The total lipin content of the scrapings from the walls of the calcified bovine lymph glands was found to be very appreciably higher than that of the caseous liquid content of these lymph gland tubercles. The lipin content of calcified human tuberculous lymph glands is low, but in this case the calcium and phosphorus values are extremely high, showing that the dry weight here is made up in large part of calcium salts. The MgO was found to hold a constant relation to the CaO and the amount present was always small; likewise, a rather definite ratio existed between the amounts of carbon dioxide and of CaO. The water-soluble fraction of the caseous liquid content of the tubercles constituted a smaller percentage of the dry weight than it did in the scrapings from the walls of these tubercles. The water-soluble materials obtained from the calcified human tuberculous glands was still much smaller in amount. In the latter case this might be due to the presence of great amounts of relatively insoluble inorganic salts.

The most extensive study of the chemical composition of tuberculous tissues has been made by Caldwell,<sup>57</sup> which is particularly

<sup>57</sup> Jour. Infect. Dis., 1919 (24), 81.

valuable in that the tuberculous tissues are compared with normal tissues. The analytical data obtained by Caldwell with bovine tuberculous tissue are given in a series of tables, for which the original article should be consulted. From them the following deductions may be drawn:

The water content of normal bovine lymph glands constitutes about 81 or 82 per cent of the moist weight. No very distinct differences are noted between peribronchial glands and those from the mesenteric region. The tubercle walls and the caseous material from lymph gland tubercles contain a lower percentage of water than does the normal tissue.

In normal bovine liver tissue, the percentage of water present is less than in the tubercle walls or the caseous material from liver tubercles. The specimens of caseous material from lymph gland and liver tubercles approach each other closely in their water content, the average being about 75 per cent for the bovine material.

The alcohol-ether-soluble substances from normal bovine lymph glands form about 24.4 per cent of the dry weight, or about 4.4 per cent of the moist weight. The walls of the lymph gland tubercles contain a distinctly larger amount of lipins than does the caseous material or the normal tissue. On the contrary, the walls of liver tubercles are poor in lipins as compared with the normal tissue, and they contain a smaller amount of fats than does the caseous material from these tubercles. When calculated on the basis of the dry weight, the caseous material from lymph gland tubercles contains a smaller percentage of lipins than does normal lymph gland tissue. When the ash is deducted, this difference disappears and the content of lipins becomes equal to or slightly greater than that of the normal tissue, but less than that of the tubercle walls. When calculated on an ash-free basis, the lipin content of the caseous material from liver tubercles is distinctly less than that of the normal tissue but greater than the lipin content of the tubercle walls. As far as lymph gland tubercles are concerned, the results obtained by these analyses seem to support the evidence furnished by staining methods that the walls of tubercles contain a larger amount of fat than does the caseous material itself. While this

does not hold true of liver tubercles, it seems quite likely that the difference is due to the more rapid formation of the tubercles in the liver tissue which is already extremely rich in fats. In all 4 specimens of caseous material the total lipins constitute a smaller percentage of the dry weight than in the normal tissues from which this caseous material originated. This shows conclusively that caseous material is not so rich in fats as it has usually been considered.

Cholesterol forms about 6.5 per cent of the lipins from normal bovine lymph glands, or about 1.5 per cent of the dry weight. The lipins from the walls of lymph gland and liver tubercles contain, in every case, 2 to 3 times as much cholesterol as do the lipins from the normal tissues. This is an actual increase also when calculated on the basis of the dry weight. The caseous material contains even a larger percentage of cholesterol than do the tubercle walls, which corresponds to the common appearance of cholesterol crystals in caseous masses. This increase of cholesterol is, of course, not peculiar to tubercles, but is a common characteristic of all necrotic tissues. For example, Dietrich<sup>30</sup> found that a kidney with total anemic necrosis contained about twice as large a proportion of cholesterol as the normal kidney, but it probably represents largely a residue from the cells that have disintegrated rather than an influx from outside, for Corper<sup>9</sup> found that in the necrotic spleen autolyzing *in vivo* the cholesterol content remains approximately constant.

Lecithin constitutes about 32 per cent of the lipin fraction of normal bovine lymph glands, or about 7.9 per cent of the dry weight; the corresponding values for normal liver are 41.2 per cent of the fats, or 14 per cent of the dry weight. The lecithin content of the fats from the tubercle walls is slightly less than that of the normal tissues, while there is a very marked reduction in the lecithin content of the lipins from caseous material of bovine origin, in contrast to the cholesterol content. In the specimen of caseous material from human lymph glands, lecithin formed 30.9 per cent of the total lipins. This finding of a decrease in the lecithin content of the fats from the caseous material harmonizes with the similar finding by Wells in his study of the fats of livers in acute yellow atrophy and in delayed chloro-

form poisoning, and also with the observation made by Griniew on the organs of tuberculous guinea-pigs. The cholesterol of the total lipins increases at about the same rate that the lecithin decreases in the tuberculous tissues, so that the sum of the two percentages remains practically constant, leaving the simple fats to form about the same percentage of the total lipins in normal and tuberculous tissues.

The iodin numbers obtained for the fats of the tuberculous specimens from lymph glands are higher than those from the normal tissues. This observation does not hold true for the liver specimens. In the latter, there is no difference noted between the iodin numbers obtained for the lipins from normal and tuberculous specimens, although the values are practically the same as those from the fats from the lymph gland tubercles.

In the residues of caseous material left after extraction with alcohol and ether, the nitrogen content remains relatively high, in fact, the reduction in nitrogen content is only slight when the calculations are made on ash-free residues. The percentage of nitrogen does not differ much from that obtained from the normal proteins of these tissues.

Even in specimens of caseous material in which there are no macroscopic evidences of calcification other than the presence of sandlike particles, calcium sometimes forms as much as 15 per cent of the residue left after extraction of the fats. In such residues, the phosphorus content may reach 9 per cent.

The total phosphorus content of the normal lymph gland residues averages twice that of the residues of the normal liver. This can be explained by the greater amount of nucleoproteins in the lymph glands. The walls of the tubercles arising in lymph glands or in bovine liver give residues which contain approximately the same amount of phosphorus. As compared with the phosphorus content of the normal tissues, the increase in the amount of phosphorus in the walls of the lymph gland tubercles is small as compared with the increase in the ash content. This is apparently due to a decrease in the nucleoproteins and their replacement by proteins poorer in phosphorus, together with the deposition of inorganic salts. In the residues from liver tubercles, the amount of phosphorus is increased to nearly 3 times the amount

in normal tissue, although the total ash content is only slightly higher than that of the walls of lymph gland tubercles. In this case there was no tissue rich in nucleins to be replaced, so that the increase in the phosphorus is due chiefly to the deposition of inorganic salts.

The amount of purine nitrogen in the walls of lymph gland tubercles is only slightly more than half that of normal lymph gland tissue, and the amount is apparently much less in the caseous material. In the residues from the walls of liver tubercles, purine nitrogen is present in only slightly higher percentage than in the normal liver.

The amount of material which enters the water solution during extraction is distinctly less from caseous material than from the residues of normal tissues.

In the water-insoluble residues of lymph glands and lymph gland tubercles, the purine nitrogen decreases with the tubercle formation and reaches a minimum in the residues of caseous material. As lymph gland tissue is replaced by fibrous tissue relatively poor in nuclein substances, a decrease in purine nitrogen would be expected in the tubercle walls. Likewise, in caseation, as the nuclear substances disappear, as shown by staining methods, a further reduction of purine content probably also occurs. From the results obtained with residues from normal liver and from liver tubercles, the tubercle walls are apparently richer in nucleoproteins than is the normal liver. A finding which at present cannot be explained, is the distinctly greater purine content of the caseous residues of liver tubercles as compared with the purines in the residues of normal liver and in walls of liver tubercles. Three closely agreeing determinations give an average value one-half more than that of the tubercle walls and approximately twice that of normal liver tissue. This does not conform with the finding of an extremely low percentage of purine nitrogen in the single specimen of caseous material from bovine lymph gland tubercles.

In addition to the bovine material, Caldwell also analyzed the caseous and partly calcified material from 3 *human* tracheo-bronchial lymph glands, which weighed only 13.5 grams and was, therefore, too little for accurate analysis. In spite of the fact

that most of the specimen formed a semifluid mass, its dry weight was 60.7 per cent of the moist weight, or the water present formed only 39.3 per cent of the original weight. From the entire specimen 0.45 grams of lipins was obtained, which is equivalent to 5.5 per cent of the dry weight, or 19.7 per cent of the ash-free residue. Lecithin was found to constitute 30.9 per cent of the total lipins, a percentage  $2\frac{1}{2}$  times as great as that in the fats from bovine caseous material. This lecithin value represents 1.7 per cent of the dry weight, or 6.1 per cent when calculated on the ash-free basis.

The iodin number of these fats was found to be 30.7, which is about the same as that obtained for the fats from normal bovine glands, but much lower than that from the single specimen of caseous material.

The alcohol-ether residue of this specimen formed 93 per cent of the dry weight, and the water-insoluble fraction 88.9 per cent. The total nitrogen determinations made on the alcohol-ether residue and on the water-insoluble fraction gave 2.46 and 2.33 per cent of nitrogen, respectively. When the ash content of these residues has been deducted, these nitrogen values become 10.93 and 10.84 per cent. This represents a reduction of the protein substances far below that seen in any of the specimens of caseous material from bovine tissues. The total phosphorus content of each of these residues was 9.25 to 9.50 per cent, while the ash constituted 77.5 to 78.5 per cent. Of this ash the calcium formed over 60 per cent of its weight. An attempt was made to evaluate the purine nitrogen, but evidence of only a trace of purines was obtained.

#### THE SPECIFIC GRAVITY OF TUBERCULOUS TISSUES

The specific gravity of the tuberculous lung is, of course, increased in proportion to the degree of consolidation and especially the amount of fibrosis. Olsho,<sup>58</sup> who has investigated this subject, states that:

As regards the lungs this method offers a more perfect mode of comparison of size of the two organs than can be obtained in any other way. In support of this statement the following cases may be cited:

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<sup>58</sup> Arch. Int. Med., 1908 (2) 171.

*Case 38.*<sup>59</sup> Patient aged twenty-six; right lung, weight 460, volume 850, specific gravity 541; chronic caseous tuberculosis; left lung, weight 780, volume 770, specific gravity 1013; chronic caseous tuberculosis and atelectasis of lower lobe.

It is clear in this case that the right was the functioning lung; less weight, greater displacement, lower specific gravity.

*Case 39.* Patient, aged thirty-five; right lung, weight 590, volume 810, specific gravity 728; edema and congestion; tuberculosis of lower lobe, hence higher specific gravity. Left lung, weight 400, volume 640, specific gravity 625; edema and congestion.

*Case 53.* Patient, aged fifty-seven; right lung, weight 560, volume 580, specific gravity 965; emphysema, congestion, healed tuberculosis; left lung, weight 190, volume 180, specific gravity 1055; atelectasis.

No description could give as adequate an idea of the conditions in this case as the figures quoted.

*Case 76.* Patient, aged twenty-four; right lung, weight 460, volume 670, specific gravity 686; acute miliary tuberculosis. Left lung, weight 460, volume 610, specific gravity 754; acute miliary tuberculosis.

The two lungs weighed the same; the right lung was larger; the left should have weighed less; the specific gravity of the left was the higher. From the figures alone it is proper to conclude that the left lung was the more involved.

*Case 88.* Patient, aged forty-six; right lung, weight 660, vol. 1200, specific gravity 500, emphysema. Left lung, weight 940, volume 1300, specific gravity 723; lobar pneumonia involving part of upper lobe; the remainder of the organ emphysematous.

*Case 108.* Patient, aged fifty; right lung, weight 540, volume 1150, specific gravity, 469; the organ apparently normal, crepitating throughout. Left lung, weight 1790, volume 1780, specific gravity 1005; lobar pneumonia; nowhere crepitant.

These few cases are sufficient to illustrate what is already known, namely, that conditions like fibrosis, atelectasis and pneumonia, increase the specific gravity of the lung. Comparison of weights, volumes and specific gravity of the two lungs gives an approximate idea of the amount of functioning tissue present in each.

The fatty liver of tuberculosis shows a low specific gravity in proportion to its fat content, but the highest specific gravity observed in the spleen was in miliary tuberculosis. The effect of tuberculosis on the specific gravity of other tissues is not described.

<sup>59</sup> Weight is given in grams, and displacement, or volume, in cubic centimeters.

## CHAPTER VI

### MINERALIZATION IN TUBERCULOSIS

#### CALCIFICATION

Calcification as it occurs in tuberculous areas does not differ from calcification in any other situation. The calcium salts are laid down in the tubercles according to the universal principle that necrotic tissues, or for that matter any non-living permeable material, whether dead tissue, avascular hyaline connective tissue, or extraneous foreign material, (e.g., silk sutures, gauze drains) which cannot be absorbed, will become impregnated with calcium salts. Why this should be so is not yet understood. Numerous attempts have been made to find some chemical substance formed in the necrotic tissues with the capacity for precipitating calcium salts out of the blood, among which phosphates from the nucleo-proteins and lecithin, and also soaps, have received most consideration.<sup>1</sup> Klotz especially has emphasized the possibility that fatty acids formed in disintegrating tissues may act as precipitants for calcium as calcium soaps, which later undergo transformation into calcium phosphate and carbonate. It is true that fatty changes often occur in degenerated tissues which subsequently become calcified, and micro-chemical reactions have been obtained in such tissues which some have interpreted as indicating the presence of soaps. There are, however, no microchemical tests that are specific for calcium soaps, and therefore it is not possible to trace the transformation of calcium soaps into inorganic salts of calcium if it does occur. Even Fischler and Gross, who believed that they could demonstrate the presence of soaps in degenerating tissues by microchemical methods, and ascribed importance to them in calcification, state that they could not find soaps in caseous areas

<sup>1</sup> The problems and literature of calcification will be found discussed more fully in Wells' "Chemical Pathology." Chap. XVII, and Arch. Int. Med., 1911 (7), 721.

and that therefore calcium soap formation cannot be an essential preliminary in calcification of tuberculosis.

Although I have repeatedly examined calcifying tissues of many origins and in all stages of calcification by chemical methods, calcium soaps could never be found in appreciable quantities. The failure to find calcium soaps in even the most rapidly calcifying materials is excellent evidence that they are not even transiently present in ordinary calcification, for simple calculation indicates that if the calcium soaps existed as such even for three or four hours in certain calcifying specimens under examination, there should have been enough present to be readily detected by chemical means. Furthermore, experiments have shown that sodium soaps in a colloidal matrix do not bind much calcium to form calcium soaps, and also that calcium soaps in a colloidal matrix are almost completely absorbed and only about 1 to 2 per cent undergoes transformation into inorganic salts. On the other hand we have the fact that calcification often occurs in tissues and materials which are not the site of fatty degeneration, and that pathological calcification seems to occur under quite the same conditions and to behave in all respects the same as normal ossification, which shows no evidence of a preliminary fatty degeneration and soap formation. Calcium deposition seems to depend rather on physical than chemical attraction, for it tends to occur in hyalin or homogeneous colloidal masses of whatever chemical nature occurring anywhere in the body. Tubercles undergo calcification probably because they present these physical conditions rather than because of any chemical constituents. The large amount of phosphoric acid that might be derived from the disintegrating nuclei of the necrotic tubercle cannot be held responsible, for I found that implanted sterile tissues rich in nuclei take up no more calcium than do tissues poor in nuclei.

The chemical composition of the calcific deposits in tuberculosis differs not at all from those occurring in other pathological conditions. Two analyses of pulmonary concretions are quoted by Ott, which, translated into percentage figures, are as follows:

|                                      |    |    |
|--------------------------------------|----|----|
| Ca phosphate.....                    | 52 | 73 |
| Ca carbonate.....                    | 13 | 6  |
| Mg salts.....                        | 2  | 1  |
| Fe <sub>2</sub> O <sub>3</sub> ..... | 3  |    |
| Silicate.....                        | 1  | 1  |
| Lipoids and fats.....                | 24 | 7  |
| Sodium salts.....                    |    | 1  |
| Organic material and loss.....       | 5  | 11 |

These analyses were made in 1837 and 1858, and their accuracy is questionable. Even more recently most of the analyses that have been made of calcium deposits have been carried out by the ashing process, which is far from accurate, since in the presence of phosphates and carbonates formed from the tissue during the ashing, the calcium salts may be converted into one or the other according to the proportions and the temperature, as I have found by direct experiment. Using a wet extraction process which eliminates this source of error, the following figures were obtained which are shown in comparison with some obtained with normal bone by other observers, who, however, used the ashing process:

#### Pathological calcification

|   | Mg <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> | CaCO <sub>3</sub> | Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> |
|---|---|-------------------|---|
| Bovine tuberculosis.....                  | 0.84  | 12.8              | 85.9  |
| Bovine tuberculosis.....                  | 0.9   | 13.1              | 85.4  |
| Bovine tuberculosis.....                  | 1.2   | 11.7              | 86.4  |
| Bovine tuberculosis (softened gland)..... | 1.5   | 7.6               | 90.6  |
| Human tuberculosis.....                   | 1.2   | 10.1              | 87.8  |
| Calcified nodule in thyroid.....          | 0.85  | 13.4              | 85.4  |
| Thrombus, human.....                      | 1.1   | 11.9              | 86.5  |

#### Normal ossification

|                           |      |       |      |
|---------------------------|------|-------|------|
| Human bone (Zalesky)..... | 1.04 | ±12.8 | 83.8 |
| Human bone (Carnot).....  | 1.57 | 10.1  | 87.4 |
| Human bone (Carnot).....  | 1.75 | 9.2   | 87.8 |
| Ox bone (Zalesky).....    | 1.02 |       | 86.1 |
| Ox bone (Carnot).....     | 1.53 | 11.9  | 85.7 |

It will be noted that there is no significant difference in the composition of calcific deposits in human and in bovine tuberculo-

sis, in spite of the fact that in bovine tuberculosis the calcification occurs in the form of minute granules scattered throughout the lesions even in an active stage, while in human tuberculosis the calcium is deposited in large masses in nearly or quite inactive tubercles.<sup>2</sup>

Analyses of a series of calcified human tuberculous lesions in this laboratory<sup>3</sup> have given the results shown on page 153.

As indicated by these figures the composition of the inorganic salts in calcified areas in the body seems to be practically the same, if not identical, whether the salts are laid down under normal conditions (ossification) or under pathological conditions; and whether in tuberculosis or some other lesion that produces avascular necrotic masses in which lime salts accumulate. This, however, is to be expected, since in each case the calcium salts must come from the blood, where they are held in true solution or in colloid suspension by the proteins, either as the carbonate and phosphate themselves, or as calcium-ion-protein compounds, or perhaps both. This suspension or solution is an unstable condition, possible only because of the extremely small proportion of calcium in the plasma (about 1:10,000), and, therefore, capable of being overthrown by increased alkalinity of the blood, changes in the proteins or CO<sub>2</sub> content, or changes in the quantity or composition of the calcium salts. It is probable, from the work of Barillé, that the calcium of the blood exists as a soluble complex double salt, tribasic calcium-carbon-phosphate (P<sub>2</sub>O<sub>8</sub>Ca<sub>2</sub>H<sub>2</sub>: 2CO<sub>2</sub>(CO<sub>3</sub>H)<sub>2</sub>Ca), this compound being possible because of an excess of CO<sub>2</sub>. Loew,<sup>4</sup> however, believes that the calcium is carried in the blood as bicarbonate. He found that a mixture of NaHCO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub> and CaSO<sub>4</sub>, dissolved by saturation with CO<sub>2</sub>, deposited pure CaCO<sub>3</sub> without any phosphate when the CO<sub>2</sub> was driven off by a current of air, but if heated, a mixture of CaCO<sub>3</sub> and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> resulted.

<sup>2</sup> Although many forms of calcified tissues, normal and pathological, contain microchemically demonstrable iron, Gierke (Virchow's Arch., 1902 (167), 318) found none in calcified tubercles in the lungs and peribronchial glands.

<sup>3</sup> Maver and Wells, Amer. Review Tuberc., 1922, (6), 649.

<sup>4</sup> Münch. med. Woch., 1914 (18), 983.

*Analysis of calcified tuberculosis lesions*

COMPOSITION OF CALCIFIC DEPOSITS

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|                                    | TOTALS AND PERCENTAGES OF DRY WEIGHT |                     |                     |                     |                     |                    | TOTALS AND PERCENTAGES OF INORGANIC MATERIAL |                     |                      |   |
|------------------------------------|--------------------------------------|---------------------|---------------------|---------------------|---------------------|--------------------|--|---------------------|----------------------|---|
|                                    | Dry weight<br>grams                  | SiO <sub>2</sub>    | Ca                  | P in-<br>organic    | Mg                  | CO <sub>2</sub>    | Total<br>inorganic                           | SiO <sub>2</sub>    | CaCO <sub>3</sub>    | Cu <sub>2</sub> (PO <sub>4</sub> ) <sub>2</sub> |
| 983. Peribronchial.....            | 1.0898                               | 0.0027 g.<br>0.24 % | 0.2736 g.<br>25.1 % | 0.1576 g.<br>14.4 % | 0.0084 g.<br>0.77 % | 0.0429 g.<br>3.9 % | 0.8829 g.<br>89.2 %                          | 0.0027 g.<br>0.30 % | 0.0973 g.<br>11.02 % | 0.7525 g.<br>85.2 %                             |
| 978. Peribronchial.....            | 0.7234                               | 0.0094 g.<br>1.2 %  | 0.0870 g.<br>12.0 % | 0.0512 g.<br>7.07 % | 0.0181 g.<br>2.5 %  | 0.0112 g.<br>1.5 % | 0.2795 g.<br>26.8 %                          | 0.0094 g.<br>3.3 %  | 0.0254 g.<br>9.0 %   | 0.1790 g.<br>64.9 %                             |
| 1013. Subpleural nodule.....       | 0.6022                               | 0.0038 g.<br>0.63 % | 0.1576 g.<br>26.1 % | 0.0060 g.<br>15.7 % | 0.0007 g.<br>0.11 % | 0.0153 g.<br>2.5 % | 0.5185 g.<br>86.1 %                          | 0.0038 g.<br>0.73 % | 0.0347 g.<br>6.79 %  | 0.4775 g.<br>92.0 %                             |
| 1028. Peribronchial.....           | 3.4263                               | 0.0119 g.<br>0.35 % | 0.6055 g.<br>20.3 % | 0.3940 g.<br>11.5 % | 0.0214 g.<br>0.62 % | 0.0004 g.<br>2.0 % | 2.1280 g.<br>62.1 %                          | 0.0119 g.<br>0.56 % | 0.1575 g.<br>7.4 %   | 1.8780 g.<br>88.3 %                             |
| 1004. Peribronchial.....           | 3.4027                               | 0.0594 g.<br>1.7 %  | 0.5984 g.<br>17.5 % | 0.3320 g.<br>9.7 %  | 0.0224 g.<br>0.65 % | 0.0791 g.<br>2.3 % | 1.8847 g.<br>55.4 %                          | 0.0594 g.<br>3.1 %  | 0.1795 g.<br>9.5 %   | 1.5645 g.<br>83.0 %                             |
| 1043. *Peribronchial.....          | 0.7446                               | 0.0051 g.<br>0.68 % | 0.1917 g.<br>25.7 % | 0.1222 g.<br>16.4 % | 0.0587 g.<br>0.68 % | 0.0222 g.<br>2.9 % | 0.6204 g.<br>84.5 %                          | 0.0051 g.<br>0.81 % | 0.0503 g.<br>7.9 %   | 0.3610 g.<br>57.5 %                             |
| 1023. Mesenteric.....              | 1.3340                               | 0.0002 g.           | 0.2900 g.<br>21.7 % | 0.1526 g.<br>11.4 % | 0.0034 g.<br>0.25 % | 0.0224 g.<br>1.6 % | 0.8021 g.<br>60.1 %                          | 0.0002 g.<br>5.0 %  | 0.0408 g.<br>93.3 %  | 0.2130 g.<br>1.5 %                              |
| 95-1922. Mesenteric.....           | 1.8258                               | 0                   | 0.4726 g.<br>25.8 % | 0.2516 g.<br>13.7 % | 0.0488 g.<br>2.6 %  | 0.0485 g.<br>2.6 % | 1.3271 g.<br>72.6 %                          | 0                   | 0.1000 g.<br>7.5 %   | 0.1771 g.<br>79.1 %                             |
| 93. Mesenteric + peribronchial.... | 4.2472                               | 0.0022 g.<br>0.04 % | 0.2326 g.<br>5.4 %  | 0.2452 g.<br>5.7 %  | 0.0250 g.<br>0.58 % | 0.0530 g.<br>1.2 % | 0.7556 g.<br>10.4 %                          | 0.0022 g.<br>0.16 % | 0.1263 g.<br>15.6 %  | 0.5424 g.<br>71.9 %                             |
| Dry pleural exudate .....          | 6.7525                               | 0.0025 g.<br>0.03 % | 0.5079 g.<br>7.5 %  | 0.6180 g.<br>9.8 %  | 0.0650 g.<br>0.96 % | 0.2391 g.<br>3.5 % | 1.9411 g.<br>28.5 %                          | 0.0025 g.<br>0.12 % | 0.5427 g.<br>27.9 %  | 1.1600 g.<br>59.7 %                             |

\* The anthracotic tissue was separated from the calcified part of the gland. Analysis showed 0.0049 gram SiO<sub>2</sub>, equivalent to 0.65 per cent in the uncalcified tissue, while the calcified material contained but 0.0002 gram SiO<sub>2</sub>.

Even were the deposits originally of composition different from the bones, this would naturally tend to become the same, since the blood continually passing between the bones and the calcified areas would bring about an equilibrium in the course of time. Calcified tubercles, in common with other calcified lesions, may eventually become transformed into true bone, not only with bone corpuscles and Haversian systems, but also with typical functioning marrow. This morphological change, however, does not involve any appreciable change in composition, for the reasons given above. Because of the extreme avascularity of calcified tubercles, however, this transformation into bone occurs less frequently than is the case with calcific deposits that are not so completely shut off from the blood vessels.

#### LUNG STONES

Zickgraf<sup>5</sup> is authority for the statement that "lung stones," which usually are the calcified centers of tubercles set free by suppuration about them, contain much silica. He gives the following figures for the composition of four such stones:

|     | WEIGHT<br><i>mgm.</i> | $\text{SiO}_2$  | $\text{CaO}$    |
|-----|-----------------------|-----------------|-----------------|
|     |                       | <i>per cent</i> | <i>per cent</i> |
| I   | 110                   | 12.45           | 10.54           |
| II  | 100                   | 5.70            | 10.4            |
| III | 225                   | 5.02            | 5.98            |
| IV  | 490                   | 4.71            | 41.08           |

Gerhardt and Strigel,<sup>6</sup> however, found reports of three analyses in the literature, in one of which no silica was found, in another a trace, in the third but 1 per cent, and their own analysis of another specimen revealed no silica.

Scherer<sup>7</sup> states that in a hospital population of 16,000 cases, chiefly pulmonary, there occurred 21 cases in which it could be definitely established that at some time "lung stones" had been coughed out and he describes several of his cases in detail. He

<sup>5</sup> Beitr. Klin. Tuberk., 1906 (5), 399.

<sup>6</sup> Beitr. Klin. Tuberk., 1908 (10), 33.

<sup>7</sup> Beitr. Klin. Tuberk., 1921 (49), 17.

reports analyses of three such specimens, which he admits are not any too accurate, with the following results:

|                          | STONE I |                       | STONE II |                       | STONE III |                       |
|--------------------------|---------|-----------------------|----------|-----------------------|-----------|-----------------------|
|                          | Totals  | Per cent of inorganic | Totals   | Per cent of inorganic | Totals    | Per cent of inorganic |
|                          | mgm.    |                       | mgm.     |                       | mgm.      |                       |
| Weight of stone.....     | 520     |                       | 976      |                       | 460       |                       |
| Calcium carbonate.....   | 204     | 57.3                  | 220      | 31.4                  | 80        | 20.5                  |
| Calcium phosphate.....   | 112     | 31.5                  | 470      | 67.1                  | 295       | 75.1                  |
| Silicic acid.....        | 17      | 4.9                   | Traces   |                       | 6         | 1.6                   |
| Magnesium salts.....     | 23      | 6.4                   | 11       | 1.5                   | 8         | 2.1                   |
| Fat and cholesterol..... | 88      |                       | 85       |                       | 32        |                       |
| Undetermined.....        | 76      |                       | 190      |                       | 34        |                       |

He comments on the low content of silicic acid in comparison with the figures published by Zickgraf. Most of the expectorated stones are very irregular in form and dissolve readily in strong acid. They probably generally come from calcified lymph glands, although they may come from calcified nodules set free in the formation of cavities in the lung tissue. He has examined several specimens of lung stones decalcified with various agents but has never been able to find either tubercle bacilli or any definite structure.

Kahle<sup>8</sup> reports finding 1.6 gram SiO<sub>2</sub> per kilo dry weight in a calcified tuberculous hilum gland of a child, but gives no figures for the other inorganic elements. He says that Weyland found in normal bovine bronchial glands 0.14 gram SiO<sub>2</sub> per kilo dry weight, in tuberculous glands 0.27 gram, and in calcified tuberculous glands 1.54 gram. The analyses of numerous specimens of calcified tuberculous lymph glands given on p. 153 also indicates that silica is not a conspicuous constituent. In the pus of tuberculous bone abscess, silica seems to be far from abundant, for Schulz<sup>9</sup> found in two specimens but 0.0532 and 0.0461 per cent of SiO<sub>2</sub>. Robin,<sup>10</sup> indeed, says that the tuberculous lung contains less

<sup>8</sup> Beitr. Klin. Tuberk., 1921 (47), 296.

<sup>9</sup> Pflüger's Arch., 1901 (84), 94.

<sup>10</sup> Treatment of Tuberculosis, Albert Robin, London, 1913.

silica than the normal lung, but apparently this is based on two analyses only.

The four following analyses are cited by Ott and von Hoesslin.<sup>11</sup>

|                               | I    | II   | III  | IV   |
|-------------------------------|------|------|------|------|
| Calcium phosphate.....        | 52.0 | 72.8 | 69.9 | 72.8 |
| Calcium carbonate.....        | 13.0 | 6.0  | 9.1  | 5.4  |
| Magnesium phosphate.....      |      | 1.0  |      | 0.9  |
| Magnesium carbonate.....      | 2.0  |      |      |      |
| Fat and cholesterol.....      | 24.0 | 7.0  | 20.1 | 7.2  |
| Other organic substances..... | 4.0  | 10.0 |      | 9.8  |

In other words, allowing for difficulties in analysis, the calcified masses set free occasionally in tuberculous lungs and peribronchial glands, exhibit quite the same composition as other areas of pathological calcification, resembling bone ash in their proportions of calcium and magnesium phosphate and carbonate, with probably admixture at times of silicates deposited from inhaled dust, and also sometimes traces of iron from old hemorrhages.

#### PNEUMONOKONIOSIS<sup>12</sup>

This condition may properly be discussed in this review, since it is so often associated in greater or less degree with pulmonary tuberculosis, and is supposed to have either a favorable or an unfavorable influence on the progress of the disease.

In a number of cases of the different forms of pneumonokoniosis quantitative analyses have been made, which may be briefly discussed as follows: Not only does the lung of every adult contain considerable amounts of coal-pigment stored up in the connective tissues (and also in the peribronchial glands), but also, which is perhaps less generally appreciated, considerable quantities of silicates are also present (chalcosis) from inhaled dust. Woskressensky<sup>13</sup> found silicates in all of 54 lungs examined, except two from infants. The lungs of individuals whose occupations do not expose them especially to dust inhalation contain

<sup>11</sup> von Hoesslin, "Das Sputum," Berlin, 1921; Julius Springer.

<sup>12</sup> See review by Landis, Jour. Indust. Hyg., 1919 (1), 117.

<sup>13</sup> Cent. f. Path., 1898 (9), 296.

increasing amounts of silicates in direct proportion to age; the silicates constitute then from 3.5 to 10 per cent of the total ash of the lungs. There is always a larger proportion of silicates in the peribronchial glands than in the lungs, constituting from 6 to 36 per cent of the ash, corresponding with Arnold's observation that in gold-beaters the glands contain more metal than the lungs. In stone-workers Schmidt found a higher proportion of SiO<sub>2</sub> in the lungs than in the glands. In normal adults the amount of coal-pigment is greater than the amount of silicates; in children the reverse is the case.

Thorel<sup>14</sup> reports that the lungs of a worker in soapstone contained 3.25 per cent of ash, including 2.43 per cent of soapstone.

In siderosis iron has been found in the lungs in proportions varying from 0.5 per cent to 7.9 per cent of the dry weight, the last amount having been found by Langguth<sup>15</sup> in the lungs of an iron miner, which contained also 11.92 per cent of SiO<sub>2</sub>.

An analysis of a lung from a knife-grinder is reported by Hodenpyle,<sup>16</sup> which gave the following results: Total weight of dried and powdered lung, 48.1009 grams; total solids, 44.7986; ether-soluble substance, 14.6017. Composition of the ether-soluble substance: free fatty acids, 7.498; neutral fats, 4.044; cholesterol, 3.037. Proteins, 15.4759; charcoal (total carbon less protein carbon), 7.198; ash, 4.2903. The composition of the ash (in grams) was as follows: K<sub>2</sub>O, 0.2167; Na<sub>2</sub>O, 0.3523; CaO, 0.0965; Fe<sub>2</sub>O<sub>3</sub>, 0.0879; Al<sub>2</sub>O<sub>3</sub>, 1.4628; SO<sub>3</sub>, 0.0704; P<sub>2</sub>O<sub>5</sub>, 0.9565; SiO<sub>2</sub>, 1.2043. The amount of emery, represented by the oxides of aluminum and silicon, made up more than one-half of the ash, and the iron constituted about one-fourth. The man had worked at the trade of knife-grinder for about fifteen years.

McCrae<sup>17</sup> has analyzed the lungs of six gold mine workers, in South Africa, finding from 9 to 21.7 grams of ash per lung, of which 29 to 48 per cent was silica; aluminum was also high, and an increased P<sub>2</sub>O<sub>5</sub> content was ascribed to the accompanying fibrosis. Klotz<sup>18</sup> found from 1.2 to 5.3 grams of free carbon in

<sup>14</sup> Ziegler's Beitr., 1896 (20), 85.

<sup>15</sup> Deut. Arch. klin. Med., 1895 (55), 255.

<sup>16</sup> Medical Record, 1899 (56), 942.

<sup>17</sup> "The Ash of Silicotic Lungs," John McCrae, Johannesburg, 1914.

<sup>18</sup> Amer. Jour. Publ. Health, 1914 (4), 887. General review on anthracosisis.

each lung, of dwellers of Pittsburg, as contrasted with 0.145 and and 0.405 grams found in the lungs of residents of Ann Arbor. Hirsch<sup>19</sup> analyzed four average Chicago lungs, finding in grams per lung:

|                    | I    | II   | III  | IV   |
|--------------------|------|------|------|------|
| Carbon.....        | 2.72 | 0.71 | 1.20 | 0.19 |
| Silica.....        | 0.18 | 0.28 | 0.69 | 0.04 |
| Calcium oxide..... | 0.45 | 0.12 | 0.02 | 0.05 |

In fibroid tuberculous lesions the content of the lungs in both inorganic elements and carbon dust is increased, for the healed and encapsulated lesions are always found more or less densely infiltrated with these substances, but we have no analytical figures on such specimens. Many students of tuberculosis have believed that such deposits have a beneficial influence on the infection, since the presence of the foreign particles seems to stimulate fibrosis,<sup>20</sup> and there is much statistical evidence that coal miners have a low incidence of pulmonary tuberculosis, in contrast with its supposed frequency in miners inhaling various forms of stone dust.

Holman<sup>21</sup> found that soot has some germicidal activity, probably due to the presence of acids and phenols, but there is no reason to believe that any such effect is exhibited by the quantities inhaled into the lungs.

Numerous attempts have been made to treat tuberculosis by administration of  $\text{SiO}_2$ , on the theory that the material will be deposited in the lungs and help in the encapsulation of the lesions. (See Silica Therapy, Chap. XVI.) One of the warm advocates of this procedure, Kühn<sup>22</sup> asserts that silicic acid is an essential constituent of tissues, and that many tissues, especially the lung, owe much of their elasticity and firmness to it, and that the encapsulating connective tissue in tuberculous lungs is rich in silicates. However, we lack accurate quantitative determina-

<sup>19</sup> Jour. Amer. Med. Assoc., 1916 (66), 950.

<sup>20</sup> See Haythorn, Jour. Med. Res., 1913 (29), 259.

<sup>21</sup> Amer. Jour. Public Health, 1913 (3), 1210.

<sup>22</sup> Münch. med. Woch., 1920 (67), 253; Zeit. f. Tuberk., 1920 (32), 321.

tions of the silica content of tuberculous and non-tuberculous lungs.

Kahle<sup>23</sup> says that study of the scars of healed pulmonary lesions showed no different figures for  $\text{SiO}_2$  than normal connective tissue. Stress is laid on the fact that calcified lesions may show silicic acid. He states that tuberculous guinea pigs given the organic silica preparation of Weyland show a markedly increased fibrosis after even a few days treatment, in which statement he is supported by Rössle, but no analytic data are given. Despite these observations, however, there seems to be little or no reason to believe either that administration of silicic acid leads to any appreciable accumulation in the lungs, or that it will in any way modify the defense reaction about tuberculous lesions. The silicates found in the lungs are probably practically all inhaled in the form of dust, although Gonnermann's figures<sup>24</sup> indicate that there is some absorption of silicate taken by mouth.

Quite the same viewpoint is supported by Robin<sup>10</sup> who holds that general and local demineralization is an important factor in the development of tuberculosis. He states that the lung loses silica as well as other inorganic components. He gives the following figures for the total inorganic matter of the lung (apparently based on one analysis for each figure): Normal lung, 12.04 gram per kilo; tuberculous lung, 7.90; unaffected part of lung in active tuberculosis, 14.27; unaffected part of lung in chronic tuberculosis, 10.25.

Landis<sup>12</sup> has reviewed the evidence on the effect of silicious dust on the lung, which seems to be conclusive that this is the form of pneumonokoniosis that leads to the greatest amount of injury to the pulmonary tissues, perhaps because of its gritty hardness, and perhaps partly because of its total insolubility. Coal dust seems to be best tolerated by the lungs, and plaster of Paris, clay and cement dust also produce much less injury than silica, quartz, flint, sandstone, emery and carborundum, as attested by the British Royal Commission on Metalliferous Mines and Quarries, and they lay particular emphasis on the harmfulness of fine crystalline silica as a cause of fibrosis.

<sup>23</sup> Münch. med. Woch., 1914 (61), 752.

<sup>24</sup> Zeit. physiol. Chem., 1917 (99), 255.

This tendency to the production of pulmonary injury may, of course, be expected equally well to facilitate infection because of the tissue injury it produces, or to tend to healing by walling off tuberculous lesions. As a matter of evidence, however, this British Commission reported that there is "an excessive mortality from respiratory diseases, and especially from phthisis," from inhalation of dust of quartz, quartzite, flint and sandstone. Also, the granite workers of Vermont are reported as having an excessive tuberculosis rate (Hoffman). The difficulty with these statements, however, lies in the fact that the chalcosis itself leads to conditions which clinically resemble fibroid phthisis, and hence it is not certain that the reputed high incidence of tuberculosis in stone workers actually exists. Landis says that in his own experience this mistake has often been made, and that when pulmonary tuberculosis does develop in persons with chalcosis, it commonly runs a chronic course, as Rössle<sup>25</sup> found for porcelain workers. Landis found, however, an actual high tuberculosis death rate in potters, which he attributes to the dust acting as a carrier of tubercle bacilli. Such attempts as have been reported in medical literature to secure curative effects through inhalation of inorganic dust by tuberculous patients or by infected animals, seem to have been unsuccessful.

Of most value are the studies of Gardner,<sup>26</sup> who caused guinea pigs to inhale Vermont granite dust and also tubercle bacilli of low virulence. The strain of bacilli used, when inhaled alone, produce pulmonary tubercles which heal completely after a short time, but in animals inhaling both dust and bacilli the lesions are more numerous, larger, with an equal amount of caseation but more fibrosis, and persist much longer than in the animals that did not receive dust. Cesa-Bianchi<sup>27</sup> also reported that guinea pigs subjected to dust inhalation were more susceptible to tubercle bacilli injected subcutaneously, which produced especially pulmonary lesions in these animals, while in the controls the lesions were especially in the spleen and liver. Although Gardner's experiments fail to show any evidence of a beneficial effect from

<sup>25</sup> Beitr. Klin. Tuberk., 1921 (47), 325.

<sup>26</sup> Amer. Review Tuberc., 1920 (4), 609.

<sup>27</sup> Zeit. f. Hyg., 1913 (73), 166.

the pulmonary fibrosis induced by the dust, and even indicate some decrease in local resistance, it cannot be said that they demonstrate what the effect of an old fibrosis in man may be on a newly acquired infection, or on the localization of existing latent infections, or on the progress of human tuberculosis which differs from that in the guinea pig in that the subject has more or less immunological defense against the bacilli.

## CHAPTER VII

### THE CHEMICAL CHANGES IN THE NON-TUBERCULOUS TISSUES OF THE TUBERCULOUS SUBJECT

From time to time analyses have been made of organs removed from the bodies of persons and experimental animals dead from tuberculosis. For the most part the results are of little significance, and the earlier contributions are reviewed by Ott.<sup>1</sup> They agree chiefly in showing a decrease in the tissue protein with an increase in the proportion of water and fat, a change which is usual in wasting diseases and in no way characteristic of tuberculosis. V. Hoesslin<sup>2</sup> has carried out analyses of water and fat content of the organs in 22 cases of pulmonary tuberculosis with various complications and his figures (see table on page 163) are probably comparable with one another, although unfortunately he analyzed organs from only one normal body for comparison, and his figures for the fat content of the liver in that case are somewhat lower than that found for the normal liver by other investigators.

This table shows the increase of the water content of the muscles, especially in cases with severe emaciation, as well as of the invisible lipin content. These changes are even more marked in the heart muscle, and with the liver in most instances. The same was the case in the lungs, although here the absolute fat increase was less marked than in the other organs, but the relative increase was about 400 per cent. Unfortunately the author does not make clear whether his lung analyses were conducted with tuberculous or non-tuberculous portions. His tables on the composition of the organs in people dying from other chronic diseases show much the same fall in proteins and increase in water and fats as in phthisis, except that there is usually somewhat less fat in the lungs, and marked fatty increase in the liver is not so often observed in the former.

<sup>1</sup> Chem. Pathol. der Tuberculose, Berlin, 1903, Chap. III.

<sup>2</sup> Deut. Arch. klin. Med., 1883 (33), 600.



Weyl and Apt<sup>3</sup> also found that the liver and heart of the tuberculous show a high fat content.

These fatty and hydropic changes in the liver in tuberculosis probably impair its functional activity somewhat, for Zandren<sup>4</sup> found in 36 advanced cases of pulmonary tuberculosis with fatty or amyloid changes in the liver, an increase of amino acid nitrogen in the urine to an average of 4.36 per cent, whereas in 13 cases without liver changes the amino-N was but 2.37 per cent of the urinary-N.

Shibata and Endo,<sup>5</sup> in a study of the comparative results of histological and chemical determination of lipins in the liver and kidney, analysed several organs from persons dying of tuberculosis. In general, they found that organs which show a high fat content chemically appear rich in fat microscopically. Their figures in per cent of the fresh weight of the organ are given in the following table:

| DIAGNOSIS                                    | ORGAN  | TOTAL*<br>LIPIN | HIGH<br>MOLEC-<br>ULAR<br>FATTY<br>ACIDS | CHOLES-<br>TEROL <sup>b</sup><br>ETC. | AMOUNT OF FAT<br>MICROSCOPICALLY |
|--|--------|-----------------|--|---------------------------------------|----------------------------------|
| Lung and intestinal tuberculosis.....        | Liver  | 2.09            | 1.71                                     | 0.38                                  | Very little                      |
| Peritonitis and intestinal tuberculosis..... | Liver  | 3.76            | 3.36                                     | 0.39                                  | Moderate                         |
| Caseous pneumonia.....                       | Liver  | 5.32            | 4.82                                     | 0.50                                  | Moderate                         |
| Lung and intestinal tuberculosis.....        | Kidney | 1.91            | 1.44                                     | 0.47                                  | Very little                      |
| Peritoneal and intestinal tuberculosis.....  | Kidney | 1.63            | 1.20                                     | 0.44                                  | Very little                      |
| Tuberculous meningitis.....                  | Kidney | 2.10            | 1.63                                     | 0.47                                  | Very little                      |
| Caseous pneumonia.....                       | Kidney | 1.53            | 1.18                                     | 0.36                                  | Very little                      |
| Pulmonary tuberculosis .....                 | Kidney | 2.17            | 1.44                                     | 0.72                                  | Very little                      |

\* Under "total lipins" is included the entire amount of substance soluble in petrol-ether according to the Kumagawa-Suto technic; under "cholesterol, etc." is included the non-saponifiable lipin fraction.

<sup>a</sup> Virchow's Arch., 1884 (95), 351.

<sup>b</sup> Acta Med. Scand., 1921 (53), 743.

<sup>c</sup> Biochem. Zeit., 1911 (37), 399.

Helly<sup>6</sup> also found that usually the histological and chemical estimations of liver fat agree, although occasionally there is a marked discrepancy. The increasing amounts of fat observed in different livers show not a regular change, but a tendency for certain proportions to appear in large numbers of cases. In his paper he gives the results of analyses of 100 livers for fat, 17 of them being from cases of tuberculosis. The average lipin content of the livers of patients dying of pulmonary tuberculosis was 42.8 per cent and of the livers of persons dying from extremely varied other conditions was 22.2 per cent, establishing chemically the common anatomical observation that in phthisis fatty changes are often found in the liver.

Fex<sup>7</sup> gives figures for the cholesterol content of the liver and kidney in one case of pulmonary tuberculosis with amyloidosis, with about normal figures for the liver (free cholesterol, 1.408; cholesterol as esters, 0.149; total cholesterol, 1.557 per cent of the dry substance), and increased amounts in the kidney (free cholesterol, 2.108; cholesterol as esters, 2.557; total cholesterol, 4.665; average normal total cholesterol in kidney about 1.6 per cent of dry weight.<sup>8</sup>

Kusonoki<sup>9</sup> states that in tuberculosis numerous cells containing microscopically demonstrable lipoids, as well as extracellular lipid particles, are found in the spleen, but he made no analyses.

In a study of the relation of the fat content of the bile to that of the liver, LeCount and Long<sup>10</sup> analysed one fatty liver from a case of pulmonary tuberculosis. Their figures are: Per cent of total dry weight as lipins, 44.1; ratio of lecithin to fat-free tissue, 1.4 per cent; ratio of cholesterol to fat-free tissue, 3.20; lipins in bile, 1.75 per cent; cholesterol in bile, 0.13 per cent. In general, the amount of lipins in the bile rises with the amount of lipins

<sup>6</sup> Ziegler's Beitr., 1914 (60), 1.

<sup>7</sup> Biochem. Zeit., 1920 (104), 82.

<sup>8</sup> Landau and McNee (Beitr. path. Anat., 1914 (58), 667), obtained the following figures for free cholesterol in the livers of three cases of pulmonary tuberculosis: 0.02, 0.34, 0.46 per cent; only the first had any cholesterol as esters (0.16 per cent), but on account of the irregularities in their results Fex considers them valueless.

<sup>9</sup> Beitr. path. Anat., 1914 (59), 564.

<sup>10</sup> Jour. Exp. Med., 1914 (19), 234.

in the liver, the proportion of lecithin in the liver remains fairly constant in relation to the fat-free liver substance, indicating that the added lipin material is infiltrated neutral fats, although there is a slight increase in cholesterol. Chauffard,<sup>11</sup> who has determined the cholesterol content of the bile in many conditions, found it usually about normal in pulmonary tuberculosis and slightly raised in tuberculous meningitis.

It is interesting to compare the marked variation in the fat and cholesterol content of the tissues from diseased persons, with the studies of Terroine and Weill<sup>12</sup> on healthy animals, which indicate that, whether starved or underfed, the amount of these lipins is practically constant in the parenchymatous organs, only the muscle showing marked fluctuations.

In the depot fat there occurs an increase of unsaponifiable material, largely cholesterol, in tuberculosis as well as in such wasting diseases as carcinoma. Wacker<sup>13</sup> gives the following figures:

|                             | AVERAGE PER CENT OF HUMAN MESENTERIC AND SUBCUTANEOUS FAT IN |              | AVERAGE PER CENT OF HUMAN SUBCUTANEOUS FAT IN |              | AVERAGE PER CENT OF HUMAN MESENTERIC FAT IN |              |
|-----------------------------|--|--------------|---|--------------|---|--------------|
|                             | Unsa-ponifi-able   | Choles-terol | Unsa-ponifi-able                              | Choles-terol | Unsa-ponifi-able                            | Choles-terol |
| 16 non carcinoma cases..... | 0.3301   | 0.1277       | 0.3090  | 0.1103       | 0.4054                                      | 0.1553       |
| 22 carcinoma cases.....     | 0.6212   | 0.2198       | 0.5489  | 0.1923       | 0.7410                                      | 0.2746       |
| 5 tuberculosis cases.....   | 0.5532   | 0.2025       | 0.4713  | 0.1591       | 0.6434                                      | 0.2403       |

Extensive analytical studies of the organs of persons dead with tuberculosis, and of inoculated animals, gave Kondratowitsch<sup>14</sup> the following results: The chemical changes were less marked in rabbits and guinea pigs than in human tissues, presumably because of the shorter duration of the process. The nitrogen content of the organs showed no considerable changes. In

<sup>11</sup> Ann. de Méd., 1920 (8), 149.

<sup>12</sup> Jour. physiol. path. gén., 1913 (15), 549.

<sup>13</sup> Zeit. physiol. Chem., 1912 (80), 383.

<sup>14</sup> Dissertation. St. Petersburg, 1914.

guinea pigs the total  $P_2O_5$  was slightly decreased in the lungs and increased in the other organs, the phosphate content of all organs was increased except in the brain, but phosphatid-phosphorus was increased in the brain and decreased in the other organs. In rabbits the phosphatid-P decreased considerably, but total and inorganic-P were not much altered. In all the human organs examined there was a noteworthy increase in total  $P_2O_5$  (liver and brain, 12 to 16 per cent; spleen, kidneys and lungs, 34 to 41 per cent). The phosphate content was much increased, especially in the spleen, kidneys and lungs. Lipoid-P was increased in the lungs, less in the brain, and decreased in the other organs. Protein-P was increased in all organs, especially the brain. Free cholesterol was increased in all organs. The cerebroside content, reckoned on the galactose, was decreased 50 per cent. In all human organs except the liver there was a slight decrease in the total nitrogen. Griniew<sup>15</sup> analyzed organs of tuberculous guinea pigs and states that the sum of all the lipoids and the lipoid-P diminishes in nearly all the organs, least in the lungs; he thinks that part of the lecithin is transformed into cephalin or a similar lipoid.

It is to be remarked that all tissue analyses need to be compared with the histological changes in the tissues, otherwise the significance of the chemical results cannot well be determined.

Analysis of the entire bodies of 2 normal and 6 tuberculous guinea pigs by Dröge<sup>16</sup> merely indicated that the tendency to water increase is exhibited by the body as a whole in tuberculosis; the fat content as a whole is decreased, despite its increase in the parenchymatous cells of the viscera; the proportion of total ash is increased, presumably because of the loss of fat and protein. The detailed results of the ash analyses are unfortunately lost, as the author died during the war before this part of his work was prepared for publication.

Kahle<sup>17</sup> reports that the pancreas of persons with active tuberculosis shows a marked reduction in its content in silica, but found no increase in the amount of  $SiO_2$  in the fibrous tissue

<sup>15</sup> Arch. Sci. biol., St. Petersburg, 1913 (17), 363.

<sup>16</sup> Pflüger's Arch. Physiol., 1916 (163), 266.

<sup>17</sup> Münch. med. Woch., 1914 (61), 752.

of tuberculous lungs. Robin<sup>18</sup> maintains that in tuberculosis there is a general demineralization, not only in the lungs but throughout the body, including the blood and even the bones. His statements seem to rest on a relatively small number of analyses. He also states<sup>19</sup> that the tuberculous lung shows a decrease in total sulphur in the more markedly involved portions.

Otalski and Biernacki<sup>20</sup> report that the organs of rabbits injected with killed tubercle bacilli (route not stated or effects described) show a decrease in the total phosphorus, in the liver a decrease in lecithin is accompanied by an increase in jecorin, and the P content of the hepatic lecithin is increased. As they used but one rabbit for control, and this apparently not kept under similar conditions of nourishment as the test animals, the value of the results reported is dubious. Schut<sup>21</sup> says that injection of tuberculin into normal guinea pigs caused hyperglycemia with decrease in the amount of glycogen in the liver, the effect of tuberculin in these respects being still more marked in tuberculous animals.

In cachexia the proportion of creatine in the muscle tissue is decreased,<sup>22</sup> and hence it presumably is lowered in advanced tuberculosis.

*Cellulose.*—A strange episode in the history of the chemistry of tuberculosis was introduced by Freund's report<sup>23</sup> that he had demonstrated the presence of cellulose in tuberculous organs, sputum, and circulating blood; his evidence being the presence of an insoluble material, not removed by ordinary solvents, and giving a sugar reaction after hydrolysis. This was confirmed by Kahrbel, while Dreyfuss sought an explanation in the presence of inhaled cellulose particles. Others believed that the tubercle bacilli formed the cellulose that was supposed to have been demonstrated, while Ruppel believed that the sugar came from chitin-

<sup>18</sup> Treatment of Tuberculosis, Albert Robin, London, 1913, English translation.

<sup>19</sup> Bull. acad. Méd., 1920 (83), 178.

<sup>20</sup> Biochem. Zeit., 1912 (41), 375.

<sup>21</sup> Beitr. Klin. Tuberk., 1915 (35), 91.

<sup>22</sup> Chisholm, Biochem. Jour., 1912 (6), 243.

<sup>23</sup> Wien. med. Jahrb., 1886, p. 375.

like substances forming the wall of the tubercle bacilli. Of course reducing carbohydrates can be formed by hydrolytic cleavage of mucin, chondrin and nucleoproteins, which would be present in abundance in tuberculous tissues, especially the lungs, and which would resist extraction by the agents used by Freund. Not so easily explained is his statement that "cellulose" is found only in tuberculous organs, and that he extracted with Schweizer's reagent a material giving exact elementary analytical figures for cellulose. This matter needs reinvestigation on the basis of present knowledge of carbohydrate chemistry.

#### THE BIOCHEMICAL BASIS FOR THE PREDISPOSITION OF THE LUNGS TO TUBERCULOSIS

Under this heading M. Weiss<sup>24</sup> advanced the theory that the low oxidative capacity of the lung determines its vulnerability for tubercle bacilli. That anatomical considerations are not chiefly responsible, he maintains is shown by the amount of lung involvement in experimental animals infected by diverse routes and methods. The familiar fact that with congenital pulmonary valve stenosis pulmonary tuberculosis is common, and in mitral stenosis it is infrequent, is to be explained by the greater blood and oxygen supply in the latter, since in the lungs the venous blood is the better oxygenated. A special susceptibility to tuberculosis was postulated by Neumann and Wittgenstein on the basis of their observation that tubercle bacilli in lung tissue *in vitro* retained their virulence longer than when they were kept *in vitro* in other tissues. This they ascribed to a low lipase content of the lung, believing that cell lipase attacks the fatty tubercle bacilli, but without advancing experimental proof of this hypothesis. As a matter of fact Sieber has shown the lung to be actively lipolytic, as also have Mayer and Morel.<sup>25</sup> It would seem much more probable that the low rate of autolysis, which is characteristic of lung tissue, is responsible for the preservation of the tubercle bacilli *in vitro*, since bactericidal substances are known

<sup>24</sup> Wien. klin. Woch., 1912 (25), 697.

<sup>25</sup> Bull. Soc. chim. biol., 1919 (1), 189.

to be produced in autolysis. Weiss called attention to the observations that (1) the lungs are among the tissues poorest in oxidase, (Ehrlich, Vernon); (2) they have the weakest accessory respiration (Batelli and Stern); (3) they are said to be the only organs that cannot reduce methylene blue (Johanssen); (4) in advanced tuberculosis urochromogen excretion is excessive, which depends on a failure to oxidize it to urochrome. He also reports the finding that leucocytes of guinea pigs exhibit the weakest oxidase (as shown by indophenol blue formation), the dog the highest. Weiss's hypothesis, however, seems poorly founded, since we have at present no reason to believe that the granules in which indophenol blue is synthesized are true oxidizing enzymes, still less that they are related to the essential oxidative capacity of the tissues (see p. 139). Furthermore, the resistance of the passive congestion lungs to tuberculosis may be equally well attributed to a high CO<sub>2</sub> content, as Corper, Gauss and Rensch<sup>26</sup> have done.

An extensive series of analyses of lung tissues have been carried out by Sieber<sup>27</sup> and her associates, which may serve as a basis of comparison with analyses of tuberculous lungs when they shall have been made. The composition as disclosed gives no apparent explanation for the supposed special vulnerability of the lungs for tubercle bacilli, which many believe is to be explained solely on anatomical grounds.

#### THE THYROID IN TUBERCULOSIS

This usually shares in the general atrophy of the organs in tuberculosis with emaciation, apparently not more or less than the other tissues. Sometimes, however, hyperplastic glands are found. The total amount of iodin in advanced stages seems to be somewhat, but not strikingly, low, which is true of other cachectic conditions. Jolin<sup>28</sup> gives the following averages in his large collection of analyses of the thyroid:

<sup>26</sup> Jour. Amer. Med. Assoc., 1921 (76), 1216.

<sup>27</sup> Zeit. physiol. Chem., 1909 (62), 251 et seq.

<sup>28</sup> Upsala Lakareforen., 1906 (11), Suppl.

|   | DRY<br>WEIGHT<br><i>grams</i> | I PER<br>GRAM<br><i>mgm.</i> | TOTAL I |
|---|-------------------------------|------------------------------|---------|
| Average of all glands.....              | 7.04                          | 1.63                         | 11.20   |
| Glands of normal adults.....            | 5.38                          | 1.56                         | 8.05    |
| Glands of 14 cases of tuberculosis..... | 4.36                          | 2.09                         | 8.72    |
| Glands of 11 cases of cancer.....       | 3.80                          | 1.55                         | 5.75    |

Hence in this series the tuberculous subjects had quite normal amounts of iodin. Among all these glands are wide extremes, the iodin content in the tuberculous glands ranging from 1.78 mgm. to 71.5 mgm. so that evidently the tuberculosis has had no noteworthy influence. Even greater variations have been found by Labb  ,<sup>29</sup> ranging from 26 mgm. for the entire gland down to little or none, but most of his figures for iodin are so low in comparison with those found by others that the accuracy of the results is open to doubt. He found that in general with chronic tuberculosis the thyroid showed increasing amounts of fibrous tissue and decreasing amounts of iodin.

In the early stage of tuberculosis of the lung a slight swelling of the thyroid is often observed clinically, not infrequently with symptoms suggesting hyperthyroidism. However, tuberculous patients without hyperthyroidism do not give the positive reaction (Goetsch test) to adrenaline that is characteristic of hyperthyroidism, although the clinical condition in tuberculosis often simulates thyroid overactivity.<sup>30</sup> It has been said that in cases of exophthalmic goiter itself tuberculosis is not often seen.

The rarity of thyroid gland tuberculosis suggests that there may be an inhibitory action of its secretions, especially of the colloid of the gland. With this in mind the colloid was pressed out of thyroid gland substance by Nathan<sup>31</sup> and graded quantities of tubercle bacilli were mixed with it and exposed for from four to twenty-four hours at 37° and then injected into guinea pigs. The injected animals, however, showed no longer duration of life than the control animals.

<sup>29</sup> Compt. Rend. Soc. Biol., 1908 (65), 371, 405.

<sup>30</sup> Nicholson and Goetsch, Amer. Rev. Tuberc., 1920 (3), 109; Heise and Brown, ibid., 1921 (4), 609.

<sup>31</sup> Zeit. Tuberk., 1921 (34), 390.

## THE ADRENALS IN TUBERCULOSIS

Chemically the adrenals are characterized by their high content in lipins, especially phospho-lipins, cholesterol and cholesterol esters, as well as the characteristic constituent, epinephrine. In normal human adrenals we have found in the total solids, 35.6 per cent ether-soluble material, of which 20.6 per cent was cholesterol and 33 per cent phospho-lipins.<sup>32</sup> The proportion of fats and lipoids varies greatly during changes of age, disease, and perhaps of function, and there are those who believe the adrenal cortex to be a chief source of the lipoids of the blood, to which much important function is ascribed in the reactions of immunity. The lipins of the adrenal cortex are said to contain little or no neutral fat, but free fatty acids which may be increased when the cholesterol decreases. Loss of body fats is not accompanied by a loss of adrenal lipoids ordinarily, although they decrease in acute infections, especially pneumonia.

According to microscopic evidence there is usually a marked decrease in the lipoid content of the adrenals in human tuberculosis (Landau<sup>33</sup>), even in cases with marked fatty deposits in the liver. The cholesterol-ester quotient of the total lipoids does not parallel the fat, being excessively low in lipoid-poor adrenals and disproportionately abundant when the lipoid content is high, whereas the free cholesterol seems to remain relatively stable in amount.<sup>34</sup> There is no constant relation between the nourishment of the body and the lipoid content of the adrenals.

In guinea pigs with experimental tuberculosis there seems to be a tendency to a slight enlargement of the adrenals, the thyroid perhaps sharing this tendency to enlargement.<sup>35</sup> Unfortunately the microscopic evidence of lipin and lipoid content of tissues does not always correspond to quantitative chemical results, especially in the adrenals (Fex<sup>7</sup>) and we can find few chemical analyses of adrenals in tuberculosis.

Landau and McNee<sup>36</sup> state that the entire cholesterol content of the adrenals is decreased in phthisis, other infections and

<sup>32</sup> Wells, Jour. Med. Research., 1908 (17), 461.

<sup>33</sup> Deut. med. Woch., 1913 (39), 546.

<sup>34</sup> Wacker and Hueck, Arch. exp. Path. Pharm., 1913 (71), 373.

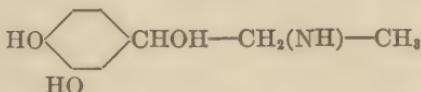
<sup>35</sup> Webb, Gilbert and Ryder, Amer. Review Tuberc., 1921 (5), 266.

<sup>36</sup> Beitr. path. Anat., 1914 (58), 607.

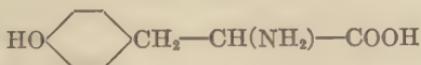
cancer, the variation being chiefly in the cholesterol esters, the free cholesterol changing less. In two cases of pulmonary tuberculosis the cholesterol esters constituted 0.41 and 0.56 per cent of the total weight of the adrenal, as compared with 1.00 and 1.47 per cent in two suicides, and with figures as high as 3.01 per cent in chronic nephritis, which is associated with the highest values. Chauffard<sup>37</sup> determined the total cholesterol content in a variety of diseases, and also found the lowest figures in infectious diseases, especially tuberculosis, and the highest in cases with arterial hypertension; thus, in tuberculosis the total cholesterol in the adrenals was generally under 1 per cent of the total weight, while in hypertension, figures of 7 to 8 per cent were common. Only in chronic fibrous pulmonary tuberculosis did they get approximately normal amounts of cholesterol, about 2.8 per cent.

Fex<sup>7</sup> gives an analysis of one adrenal in a case of tuberculosis, which had a very low figure for cholesterol esters with a normal figure for free cholesterol.

The medulla is characterized by the remarkably active internal secretion, epinephrine, which it always contains in greater or less amount. Presumably epinephrine, of which the formula is



is derived from the aromatic radical of the proteins, its close relationship to tyrosine being seen when the formula of the latter is compared.



It is to be borne in mind that the formation of epinephrine is not limited to the adrenals, but that other islands of chromaffin sympathetic tissue can do the same,<sup>38</sup> which explains the observed discrepancies between the anatomic changes in the adrenals and the clinical manifestations of a deficiency in epinephrine.

<sup>37</sup> Chauffard, Laroche and Grigaut, Compt. Rend. Soc. Biol., 1914 (76), 529; Ann. de Méd., 1920 (8), 149.

<sup>38</sup> See Vincent, Proc. Roy. Soc., B, 1908 (82), 502.

According to Goldzieher<sup>39</sup> the normal human adrenals contain together about 4 mgm. epinephrine, which may be increased in conditions with high blood pressure, such as arteriosclerosis, and nephritis, in which he found an average of 5.8 mgm.; and in septic conditions with low pressure he found it reduced to an average of 1.5 mgm. The human adrenal contains no epinephrine before birth.<sup>40</sup> Ingier and Schmorl,<sup>41</sup> using both morphological and chemical methods, found a gradual increase in the epinephrine content of normal glands from birth to the ninth year, after which it remains practically constant at about 4.5 mgm. (males 4.4, females 4.71 mgm.). In their 517 cases of all sorts of diseases were 70 of pulmonary tuberculosis, with an average of 4.06 mgm., or near the normal figure. Of these 70, 34 had less than normal and 29 had normal amounts of epinephrine, with extremes of 11.5 mgm. and 0.68 mgm. In 12 cases of tuberculous meningitis the average epinephrine content was 5.15 mgm.; in 10 other cases of tuberculosis of various locations, the average was 3.42 mgm.

Similar results were obtained by Lucksch,<sup>42</sup> who gives the following figures in cases of tuberculosis, together with his normal averages:

|                                  | NUMBER<br>OF CASES | AVERAGE EPINEPHRINE        |
|----------------------------------|--------------------|----------------------------|
| Miliary tuberculosis in infant.. | 3                  | 0.23 mgm. normal 0.42 mgm. |
| Generalized tuberculosis .....   | 3                  | 2.53 mgm. normal 4.29 mgm. |
| Pulmonary tuberculosis.....      | 35                 | 4.45 mgm.                  |
| Tuberculous pleuritis .....      | 1                  | 5.25 mgm.                  |
| Tuberculous peritonitis.....     | 1                  | 1.98 mgm.                  |
| Tuberculous meningitis.....      | 7                  | 5.80 mgm.                  |
| Tuberculosis universalis.....    | 11                 | 4.54 mgm.                  |

The amount of chromaffin substance and epinephrine do not always run parallel, although Borberg<sup>43</sup> found a close parallelism. Elliott<sup>44</sup> found a low epinephrine content in acute infectious

<sup>39</sup> Wien. klin. Woch., 1910 (23), 809.

<sup>40</sup> Moore and Purinton, Amer. Jour. Physiol., 1900 (4), 51; Julian Lewis, Jour. Biol. Chem., 1916 (24), 249.

<sup>41</sup> Deut. Arch. klin. Med., 1911 (104), 125.

<sup>42</sup> Virchow's Arch., 1917 (223), 290.

<sup>43</sup> Skand. Arch. Physiol., 1912 (27), 341; 1913 (28), 91.

<sup>44</sup> Quart. Jour. Med., 1914 (8), 47.

diseases, and especially low in acute cardiac failure associated with great mental distress; he did not find any increase in the epinephrine in nephritis or in any other disease. In tuberculosis with high fever or septic infections the cortical lipoid was reduced in amount, but in chronic cases there was more lipoid no matter how much the emaciation; in two acute cases the epinephrine content was 1.4 and 2.14 mgm.

#### ADDISON'S DISEASE<sup>45</sup>

As this condition is most frequently the result of tuberculosis of the adrenals its chemical aspects will be discussed briefly. The profound deficiency in the pressor principles evident in the manifestations of Addison's disease implies loss of function, not only of the adrenal medulla, but also of the rest of the chromaffin tissues which produce this same sort of material, and so it is possible to have any amount of destruction of the adrenals without Addison's disease, if there is sufficient compensation by the other chromaffin structures; or, conversely, Addison's disease may occur when the adrenals seem morphologically little altered, which is the condition in about 10 per cent of all cases. In typical cases, however, the adrenals have been found entirely devoid of epinephrine,<sup>46</sup> and usually the structural alterations are conspicuous. While some have held that the destruction of the adrenal cortex is of importance in Addison's disease, this does not seem to have been conclusively demonstrated.

The pigmentation of the skin<sup>47</sup> has not yet been explained, but in view of the fact that oxidizing enzymes readily convert epinephrine, tyrosine, and related aromatic substances into pigments, and that in Addison's disease we have a deficiency in a tissue which is known to be concerned in the metabolism of aromatic compounds, it seems probable that the pigmentation is the result of this defective metabolism of the chromogenic aromatic compounds. In support of this view is the observation of Bittorf<sup>48</sup>

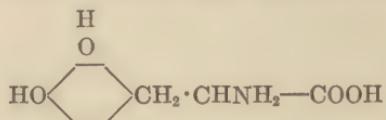
<sup>45</sup> Literature on Chemistry, by Eiselt, *Zeit. klin. Med.*, 1910 (69), 393.

<sup>46</sup> Ingier and Schmorl,<sup>41</sup> Elliott.<sup>44</sup> In one case Lucksch<sup>42</sup> found 0.80 mg. epinephrine in the tuberculous adrenals.

<sup>47</sup> According to Straub (*Deut. Arch. klin. Med.*, 1909 (97), 67) pigmentation may occur within 17 days after thrombosis of the adrenal vein.

<sup>48</sup> *Arch. exp. Path.*, 1914 (75), 143; *Deut. Arch. kl. Med.*, 1921 (136), 314.

that the skin of persons with Addison's disease has an augmented power of oxidizing epinephrine and tyrosine to pigmented substances. Bloch<sup>49</sup> believes the pigmentation to result from the presence of excessive quantities of 3,4-dioxyphenylalanine,



which may be a precursor of epinephrine, and which is oxidized to a melanin by special oxidizing enzymes ("dopaoxidase") present in the skin. As the "dopa" reaction is given by skin that has been boiled, and as it is not specific, since other substances are also oxidized, it probably is not a true enzyme action. Nevertheless, Hendorfer<sup>50</sup> also looks upon the pigmentation of the skin in Addison's disease as an effort to compensate for the failure of the adrenals to dispose of the aromatic radicals. Until the pigment of Addison's disease has been isolated and analyzed, however, these hypotheses will probably remain unproved. Addison's disease may occur without pigmentation.

That there is a deficiency in the formation of epinephrine is attested by the low blood pressure and general low tone of the unstriated muscle tissue. Carbohydrate metabolism is also altered, Porges<sup>51</sup> having found hypoglycemia in Addison's disease; however, hypoglycemia is not present in all cases, even with severe manifestations.<sup>52</sup> There is an increased tolerance to large doses of glucose given by mouth<sup>53</sup> and even injections of epinephrine do not cause glycosuria despite the production of marked hyperglycemia.<sup>54</sup>

Whether the adrenals exert a detoxicating effect, and the symptoms of the disease are partly the result of an autointoxication of some sort, is at present unknown, although this idea has

<sup>49</sup> Zeit. exp. Med., 1917 (5), 179; Arch. f. Dermatol., 1917 (124), h. 2.

<sup>50</sup> Münch. med. Woch., 1921 (68), 266.

<sup>51</sup> Zeit. klin. Med., 1909 (69), 341; also Bernstein, Berl. klin. Woch., 1911 (48), 1794.

<sup>52</sup> Broekmeyer, Deut. med. Woch., 1914 (40), 1562.

<sup>53</sup> See Myers, Jour. Lab. Clin. Med., 1920 (5), 640.

<sup>54</sup> See Leschcziner, Virchow's Arch., 1917 (19), 67.

often been advanced. The general metabolism of Addison's disease shows no very striking or characteristic changes, over and above those associated with the emaciation.<sup>55</sup> Wolf and Thacher<sup>56</sup> found a decrease in endogenous creatine and purine excretion, and some evidences of acidosis toward the end of the disease; deaminizing power and oxidation of cystine sulphur to SO<sub>4</sub> were not impaired. Eiselt<sup>45</sup> believes that there is a toxicogenic loss of tissue with increased excretion of uric acid as well as other nitrogenous urinary constituents.<sup>57</sup> He also described a considerable increase in the urinary excretion of neutral sulphur in the late stages of the disease.

In two cases of Addison's disease, Frothingham<sup>58</sup> found the CO<sub>2</sub> tension of the alveolar air just below the lower limit of normal; in one of these cases the sodium bicarbonate test showed an increased tolerance, and in both the urine showed an H-ion concentration below pH=4.7.

Examination of the blood in one case of Addison's disease by Bloor<sup>59</sup> showed the total ether-soluble material high but within normal limits, the fat slightly high in the plasma, and the lipoids practically normal. According to Lampé<sup>60</sup> the blood of patients with Addison's disease gives a specific Abderhalden reaction with adrenal tissue as substrate.

Administration of adrenal tissue and extracts, or epinephrine, whether by mouth or subcutaneously, is not effective in ameliorating the course of Addison's disease, at least in most cases. Thus, in 97 cases collected by Adams,<sup>61</sup> adrenal treatment caused some improvement in 31, 43 were not benefited, 7 became worse, while 16 were described as permanently improved. The most favorably affected is usually the muscular and gastro-intestinal asthenia, while the pigmentation is not usually altered. There is little effect on metabolism.<sup>55</sup>

<sup>55</sup> Beutenmüller and Stoltzenberg, Biochem. Zeit., 1910 (28), 138.

<sup>56</sup> Arch. Int. Med., 1909 (3), 438.

<sup>57</sup> Corroborated by Leschziner.<sup>54</sup>

<sup>58</sup> Arch. Int. Med., 1916 (18), 717.

<sup>59</sup> Jour. Biol. Chem., 1916 (25), 577.

<sup>60</sup> Münch. med. Woch., 1914 (51), 462.

<sup>61</sup> Practitioner, 1903 (71), 472.

## AMYLOID INFILTRATION

Although not a specific feature of tuberculosis, amyloidosis is most often observed in man as a result of chronic ulcerative tuberculous lesions.<sup>62</sup> Thus, of 105 cases of amyloidosis occurring in 6320 autopsies, all but seven were the result of tuberculosis (Zahn).<sup>63</sup> Of 201 cases analyzed by Dickinson,<sup>64</sup> 53 were in pulmonary tuberculosis and 62 in bone suppuration, generally tuberculous. Of 279 cases of amyloidosis observed in 18,153 autopsies in Vienna, 221 occurred in 3716 cases of tuberculosis (Schrötter) most often when associated with intestinal ulceration, 67.7 per cent of the cases in this series showing both lung and intestinal lesions.<sup>65</sup> How the changes of suppuration and other forms of tissue disintegration (syphilis, cancer) cause the deposition of amyloid is at present entirely unknown, despite the existence of considerable information concerning its chemical nature.

Krawkow<sup>66</sup> in 1897, gave us the first good idea of the composition of amyloid substance through his amplification of Oddi's observation that amyloid organs contain *chondroitin-sulphuric acid*, finding that amyloid is a compound of protein with this acid, similar to nucleoprotein, which is a compound of nucleic acid and protein. This work has received general acceptance, although a later paper by Hanssen<sup>67</sup> reports a study of amyloid material isolated in pure condition from sago spleens by mechanical means, which contained *no* chondroitin-sulphuric acid, although the amyloid organs taken *in toto* did contain an excess of sulphur as sulphate. This important contradiction to prevailing ideas apparently has not been subjected to investigation by others, with the exception of a casual remark by Mayeda<sup>68</sup> that a preparation of amyloid which he had made did not yield sulphuric acid, and the failure

<sup>62</sup> An excellent review of the older work and theories is given by von Schrötter in Ott's *Chemie der Tuberkulose*, Chap. IV.

<sup>63</sup> *Munch. med. Woch.*, 1902 (49), 49.

<sup>64</sup> Albutt's *System of Medicine*. 1901 (3), 255.

<sup>65</sup> See also Sorgo, *Zeit. klin. Med.*, 1907 (61), 250.

<sup>66</sup> *Arch. exp. Path. u. Pharm.*, 1897 (40), 196.

<sup>67</sup> *Biochem. Zeit.*, 1908 (13), 185.

<sup>68</sup> *Zeit. physiol. Chem.*, 1909 (58), 475.

of Eppinger<sup>69</sup> to find either sulphur or chondroitin-sulphuric acid in a specimen of localized amyloid in the liver. Schmiedeberg<sup>70</sup> does much speculation on the relation of chondroitin-sulphuric acid, or of hyaloidin (the carbohydrate complex of the mucoids), to amyloid, but as he made no investigations of his own in this field his discourse adds nothing to existing knowledge.

Amyloid, when isolated, is a nearly white powder, which is easily soluble in alkalies, but slightly in acids, and is very resistant to pepsin digestion. The elementary percentage composition was found by Krawkow to be approximately as follows: C, 49-50; H, 6.65-7; N, 13.8-14; S, 2.65-2.9; P, in traces only.

Quite similar analytic results have been obtained by Neuberg<sup>71</sup> who corroborated Krawkow's finding of a body of apparently similar composition in the normal aorta. Neuberg has studied especially the protein constituent of the amyloid compound, and found it characterized by a high proportion of diamino-nitrogen,<sup>72</sup> as compared with most proteins, as shown in the following table giving the percentage of the total N contained in each of the three forms, amid-nitrogen (ammonia), monamino-acids, and diamino-acids:

|                      | MONAMINOACID<br>NITROGEN | DIAMINOACID<br>NITROGEN | AMID<br>NITROGEN |
|----------------------|--------------------------|-------------------------|------------------|
| Liver amyloid.....   | 43.2                     | 51.2                    | 4.9              |
| Spleen amyloid.....  | 30.6                     | 57.0                    | 11.2             |
| Aorta "amyloid"..... | 54.9                     | 36.0                    | 8.8              |
| Gelatin.....         | 62.5                     | 35.8                    | 1.6              |
| Casein.....          | 76.0                     | 11.1                    | 13.4             |

The variations in the composition of the different amyloids, as shown in the above table, indicate that the protein group may vary in different organs in different cases, and also indicate that the "amyloid-like" substance of normal vessels is not the same

<sup>69</sup> Biochem. Zeit., 1921 (127), 107.

<sup>70</sup> Arch. exp. Path. u. Pharm., 1920 (87), 60.

<sup>71</sup> Verh. Deut. Path. Gesell., 1904 (7), 19.

<sup>72</sup> Corroborated by Jackson and Pearce (Jour. Exp. Med., 1907 (9), 520), but not by Mayeda (Zeit. physiol. Chem., 1909 (58), 469), who found histidine, which Neuberg had missed, and a lower arginine and lysine content than histon requires.

as the pathological substance. Corresponding variations were found in the apportionment of the sulphur between that which is in the form of oxidized sulphur and the unoxidized sulphur. The proportion of the different amino-acids in the protein constituent of amyloid is strikingly like that of thymus histon, and entirely dissimilar to the apparently closely related elastin, as shown by the following table:

|                        | CLEAVAGE PRODUCTS (IN PERCENTAGES) |         |               |
|------------------------|------------------------------------|---------|---------------|
|                        | Amyloid                            | Elastin | Thymus histon |
| Glycine.....           | 0.8                                | 25.8    | 6.5           |
| Leucine.....           | 22.2                               | 45.0    | 11.8          |
| Glutaminic acid.....   | 3.8                                | 0.7     | 3.7           |
| Tyrosine.....          | 4.0                                | 0.3     | 5.2           |
| <i>a</i> -Proline..... | 3.1                                | 1.7     | 1.5           |
| Arginine.....          | 13.9                               | 0.3     | 14.5          |
| Lysine.....            | 11.6                               | ....    | 7.7           |

This carries out the resemblance of amyloid to nucleoproteins, and, likewise, Neuberg found amyloid very slowly digested by pepsin and much better by trypsin, although less rapidly than simple protein; it is also destroyed by autolytic enzymes, for amyloid tissues readily undergo autolysis.<sup>73</sup> Neuberg considers, from the above results, that amyloid is probably a transformation-product of the tissue protein, similar to the transformation of simple proteins into protamins that occurs in the testicle of spawning salmon as they go up the streams, as shown by Miescher's classical studies. Mayeda<sup>72</sup> found histidine in liver amyloid, (although Neuberg had missed it in his preparation), and much less arginine and lysine, and concludes that the hexone base content of the amyloid is similar to that of the liver itself. Eppinger,<sup>69</sup> however, found neither histidine nor cystine in a local amyloid tumor of the liver, but found 14.67 per cent of arginine, 4.34 per cent of lysine, and the strikingly high figure of 12.34 per cent for tyrosine. It would seem probable, in view of the wide variations in the analytical results, that pure preparations of amyloid have not been the subject of analysis.

<sup>73</sup> Concerning the absorption of amyloid see Dantchokow, Virchow's Archiv., 1907 (187), 1.

Raubitschek<sup>74</sup> found that isolated amyloid, when used for immune reactions, behaved like a specific protein, different from the normal proteins of the animal from whence it came and apparently biologically the same in different species. (This observation awaits confirmation.)

Krawkow considers that amyloid differs from normal chondroitin-sulphuric acid compounds, such as cartilage, in that in the latter the acid radical is in a loose combination with the protein, while in amyloid the combination is a very firm one, perhaps in the nature of an ester. The occurrence of the typical amyloid reaction in what appears otherwise to be normal cartilage, occasionally observed in senile tissues, may be due to the transformation of loosely bound into firmly bound chondroitin-sulphuric acid. In any event amyloid is not essentially a pathological product, but rather a slightly modified normal constituent of the body. However, in view of the contradictory results of Hanssen and Mayeda, as yet uncontested, the chemical nature of amyloid must be considered as undetermined. Schmiedeberg makes the suggestion that it is merely serum globulin which has failed of cleavage, and thus not being capable of being metabolized, is deposited in the tissues; but for this hypothesis he has no evidence except a similarity in Hanssen's figures for the elementary analysis of amyloid with those for serum albumin. An important consideration is that amyloid deposition occurs under similar conditions in all sorts of animals, including birds; it is very often found in the livers of antitoxin horses, and mice are especially prone to a severe amyloidosis after relatively slight and brief infectious processes.<sup>75</sup>

*Staining properties.* The classical reaction for amyloid is its staining a reddish brown when treated with iodin (best as Lugol's solution) in the fresh state. Such stained specimens, if afterward treated with dilute sulphuric acid, usually become blue or greenish, but may merely turn a deeper brown. Occasionally old compact amyloid may stain bluish or green with iodin alone. The iodin reaction disappears in specimens that have been kept for some time in preserving fluids, or in tissues that have

<sup>74</sup> Verh. Deut. Path. Gesell., 1910 (14), 273.

<sup>75</sup> See Finzi, Lo Speriment., 1911 (65), 483; Davidsohn, Virchow's Arch., 1908 (192), 226.

become alkaline, and is generally less persistent than the metachromatic staining by methyl-violet or methyl-green, which color the amyloid red. Occasionally an otherwise typical amyloid will fail to react to iodin, but will stain well with methyl-violet. All these variations may occur in different specimens from the same body, and the blue iodin-sulphuric acid reaction is usually given well only by splenic amyloid. These variations probably depend upon the age and stage of development of the amyloid, or upon secondary alterations, and are perhaps related to Neuberg's observations on the difference in composition of amyloid of different origins.

Krawkow studied these reactions with pure, isolated amyloid, and found evidence that the iodin reaction depends upon the physical properties of the amyloid, while the methyl-violet stain is a chemical reaction, and hence the iodin reaction is much the more readily altered or lost. As Dickinson<sup>64</sup> says, amyloid stains with iodine simply as if it absorbed the iodin more than does the surrounding tissue. Krawkow believed that the methyl-violet reaction is due to the dye forming a compound with the chondroitin-sulphuric acid, for he found that these substances unite with one another to form a rose-red precipitate. Hanssen, however, holds that the dyes react with the protein, the iodin with some other, unknown labile substance. Schmidt found that implanted pieces of amyloid lost their iodin reaction as they underwent digestion, while the methyl-violet reaction was still very distinct.<sup>76</sup> It is evident, therefore, that iodin is not by itself a specific stain for amyloid, especially as glycogen gives a similar reaction,<sup>77</sup> while true amyloid may not react. Leupold<sup>78</sup> summarizes his investigations as follows: Amyloid is a complex of different substances which are differentiated by micro-chemical reactions. The protein ground substance of the amyloid is refractory to the typical amyloid reactions. The group which is responsible for the methyl-violet reaction is intimately combined with this protein substance and is separated from it only by the action of alkali. The groups which give respectively the iodin and the iodin sulphuric acid reactions are closely related to each other. Nevertheless the iodin sulphuric acid reaction is a completely independent one and is not a modification of the iodin reaction. The occurrence of different colors in the iodin-sulphuric acid reaction depends upon different degrees of oxidation. Amyloid is an emulsion colloid in the gel state. After oxidation with potassium permanganate it is soluble in ammonia, NaOH and Ba(OH)<sub>2</sub>. Conjugated sulphuric acid plays an important part in the production of amyloid in the organism. The existence of large amounts of conjugated sulphuric

<sup>76</sup> Litten (Verh. Deut. Path. Gesell., 1904 (7), 47) states that thionin and kresyl-violet are the most specific stains for amyloid, which they color blue; whereas methyl-violet stains red not only amyloid but also mucin, mast cell granules, and the ground substance of cartilage. V. Gieson's stain usually colors amyloid pale yellow, and hyalin red.

<sup>77</sup> See Wichmann, Ziegler's Beitr., 1893 (13), 487.

<sup>78</sup> Beitr. path. Anat., 1918 (64), 347.

acid produces amyloid which gives the iodin reaction. The methyl-violet reaction also depends on the presence of conjugated sulphuric acid; however, for its production there must probably occur a reduction in the amyloid protein. The group which gives the iodin-sulphuric acid reaction occurs through decomposition and perhaps does not depend upon the sulphuric acid.

### *The origin of amyloid.*

This question has not been at all cleared up as yet by the advances made in our knowledge of the chemistry of amyloid substance. The fact that chondroitin-sulphuric acid is a characteristic constituent suggests that this body may be liberated in considerable amount during the destructive processes to which amyloidosis is usually secondary; this idea is further supported by the fact that amyloidosis occurs particularly after chronic suppuration in bone and lungs, both of which tissues, according to Krawkow, contain chondroitin-sulphuric acid. This idea was not substantiated, however, by the experiments made by Oddi and by Kettner,<sup>79</sup> who fed and injected into animals large quantities of the sodium salt of chondroitin-sulphuric acid without producing amyloid changes. Unpublished experiments of the writer with the same material, as well as with ground-up cartilage and with mucin, were equally unsuccessful. Likewise mice injected by Strada<sup>80</sup> with the nucleoprotein of pus, the so-called pyin, or with chondroitin-sulphuric acid, did not develop amyloidosis. Oestreich<sup>81</sup> injected cancer patients with chondroitin-sulphuric acid for therapeutic purposes, but no amyloidosis resulted. As it is possible to cause amyloidosis experimentally in animals, especially chickens and rabbits, by causing protracted suppuration or chronic intoxication with bacterial filtrates, these negative results speak strongly against the idea of a transportation of chondroitin-sulphuric acid, but do not determine it finally.<sup>82</sup> They may also,

<sup>79</sup> Arch. exp. Path. u. Pharm., 1902 (47), 178.

<sup>80</sup> Biochem. Zeit., 1909 (16), 195.

<sup>81</sup> Zeit. Krebsforsch., 1911 (11), 44.

<sup>82</sup> Also they do not support the hypothesis of Frank (Münch. med. Woch., 1916 (63), 452) that amyloidosis results from infection with an organism related to the pneumobacillus, the mucinous capsule of which together with the coagulation necrosis of the tissues constitutes the amyloid.

with propriety, be used in support of the statement of Hanssen that amyloid does not contain chondroitin-sulphuric acid. Leupold<sup>78</sup> advances the following hypothesis: In chronic suppuration a soluble protein circulates in the blood, which stimulates the formation of "defensive ferments." This protein substance, under certain conditions, is deposited in organs where large amounts of sulphuric acid occur. For the development of amyloid there are necessary three factors: A preformed protein, an increased amount of conjugated sulphuric acid, and an inefficiency of the amyloid-filled organ to eliminate the increased amount of conjugated sulphuric acid.

Dietl<sup>83</sup> reports that cases of tuberculosis with amyloidosis may show demonstrable amounts of chondroitin-sulphuric acid in the urine before there is any albuminuria, although later the urine in renal amyloidosis usually contains much euglobulin and fibrinogen (Joachim and Wallerstein). If Dietl's observation is true it supports the hypothesis that amyloidosis is a form of infiltration with chondroitin-sulphuric acid or a compound thereof.

<sup>83</sup> Beitr. Klin. Tuberk., 1922 (51), 18.

## CHAPTER VIII

### CHEMISTRY OF THE BLOOD IN TUBERCULOSIS

#### GENERAL FEATURES

The careful review of this subject by Moraczewski in Ott's book concludes with the following summary of the results obtained up to 1902:

If we take the chief results together we find in the blood of the tuberculous a decrease in the coloring matter without corresponding decrease in the iron, decrease in serum protein, an increase in the potassium salts and the appearance of cellulose. In the first stage there is an increase in the fibrin, which decreases in the second and third stages. With the progress of the disease, iron, potassium and phosphate decrease steadily and the sodium salts steadily increase. The second stage of the disease leads to a concentration of the blood.

Of these observations, that concerning cellulose is based on Freund's observations, which are doubtful to say the least. To take up somewhat more in detail the observations on which the other conclusions are based, the following may be mentioned as perhaps the most significant of the findings of the earlier investigations.

Grawitz divided tuberculosis into three stages. In the first there is a slight anemia and the blood findings are those usual in anemia, accompanied by those of acute infectious diseases. There is a decrease in solids, the blood being diluted without increase of the salts; sometimes the fibrin is increased; the color index is normal. The second stage shows concentration of the blood with normal color index, there being an increase of solids in the organic fraction, but not of the salts. In the third stage the blood shows the changes of severe anemia and cachexia, often resembling pernicious anemia; the color index is more than one; there is now a decrease in solids and increase in salts. These findings are, however, not characteristic for tuberculosis.

While some authors have believed that there is an increase in the quantity of blood, probably there is no actual increase. In the second stage, indeed, there is an actual decrease in the quantity of blood and a lower osmotic pressure.

Several observers agree that the per cent of solids is increased in the second stage and that in the third stage the amounts may or may not be greater than in the first stage. A part of the table cited by Moraczewski follows:

| OBSERVER  | STAGE I<br>H <sub>2</sub> O PER M.        | STAGE II<br>H <sub>2</sub> O PER M.                | STAGE III<br>H <sub>2</sub> O PER M.  |
|-----------|---|--|---|
| Appelbaum | 805                                       | 780-760  | 850-820   |
| Grawitz   | 813.4                                     | 789.1  | 791.0 without fever<br>803.0 with fever   |
| Strauer   | 813.4<br>808.2<br>806.0<br>815.5<br>803.3 | 772.5<br>799.0<br>799.7<br>789.1<br>798.6<br>766.0 | 792.0<br>803.0<br>814.8<br>809.9<br>818.5<br>813.2<br>826.2<br>835.7<br>859.6<br>850.0<br>826.1<br>855.7<br>875.8 |

As seen from the foregoing, the water content of the blood is variable in tuberculosis. While many pathologists have described the tissues of the tuberculous cadaver as dry, analyses of the tissues have actually shown that the proportion of water is increased. Meyer-Bisch<sup>1</sup> has discussed at length the water metabolism of the tuberculous subject. He found that while the normal individual at rest in bed adds 500 to 1500 grams weight during the day, presumably through retained water, yet at the same time the proportion of total protein in the blood increases somewhat; the hemoglobin shows little alteration. By the next morning the figures

<sup>1</sup> Deut. Arch. klin. Med., 1920 (134), 185.

have returned to the figures of the previous morning. Tuberculous patients in the second stage of the disease showed a similar daily rise and fall in weight, but the serum protein concentration showed a reverse curve, being lower in the evening; i.e., in tuberculosis the blood plasma is diluted in the evening. Patients with early tuberculosis have usually the normal serum concentration curve, but a tuberculin reaction in such a patient may produce the reversed curve seen in advanced tuberculosis. In many cases the tuberculin reaction is followed by a rapid rise in weight from water retention.<sup>2</sup>

The specific gravity varies, being generally higher in the second stage and lower in the third. Von Eisler and Laub<sup>3</sup> state (without giving the reference), "we have shown in earlier publications, in agreement with Czepai and v. Torday, that in tuberculosis there is on the average a higher viscosity of the blood serum than in health or in other diseases." Gullbring<sup>4</sup> found that the viscosity tends to vary directly with the leucocyte count.

There are many contradictions in reports on the solid contents of the blood, which presumably means that in different stages of the disease the blood composition varies. Moraczewski believes in Grawitz' explanation of a lymphagogue action of the tuberculosis poison as explaining the water loss which causes the concentration commonly found in the second stage.

Rzentkowski,<sup>5</sup> in a paper somewhat more recent than those under discussion, gives the following figures:

| CONDITION                                    | TOTAL N<br><i>volume per cent</i> | RESIDUAL N<br><i>volume per cent</i> | SOLIDS<br><i>weight per cent</i> |
|--|-----------------------------------|--------------------------------------|----------------------------------|
| Normal average.....                          | 3.5183                            | 0.0469                               | 21.233                           |
| 1. Early pulmonary tuberculosis...           | 2.9008                            | 0.057                                | 17.84                            |
| 2. Severe active pulmonary tuberculosis..... | 3.068                             | 0.025                                | 19.62                            |
| 3. Severe active pulmonary tuberculosis..... | 2.366                             | 0.059                                | 14.887                           |
| Average in 3 cases of tuberculosis.          | 2.745                             | 0.047                                | 17.449                           |

<sup>2</sup> Zeit. exp. Med., 1921 (25), 295.

<sup>3</sup> Wien. klin. Woch., 1913 (26), 968.

<sup>4</sup> Beitr. Klin. Tuberk., 1914 (30), 1.

<sup>5</sup> Virchow's Arch., 1905 (179), 411.

Evidently in this series, at least, there was a marked dilution of the blood, with the non-protein nitrogen remaining about normal.

The observations on the alkalinity of the blood, by the older methods, are valueless, but it was supposed that there is a decreased alkali content despite an increased quantity of sodium and potassium salts.

Many varying figures are given for the chlorine, phosphorus and iron of the blood, and an excess of potassium and a lack of sodium is described by Biernacki. There seems to be no doubt, according to Moraczewski, that there is a steady decrease in the blood potassium, but the sodium chloride seems to be increased in the late stages. The iron and phosphorus run parallel to the potassium.<sup>6</sup> The red corpuscles are low in hemoglobin, thus resembling chlorosis. Moraczewski considers that this fact, together with the behavior of potassium and phosphorus, is characteristic for the blood in tuberculosis. The calcium content of the blood is generally within normal limits<sup>7</sup> (see discussion of Mineral Metabolism, Chap. XI). There may be a hypochloremia, which generally parallels the severity of the disease according to Boenheim.<sup>8</sup>

*Blood proteins.* Much work has been done on the fibrin content of the blood. Some maintain that there is an increase in the amount of fibrin even when the total solids are reduced. The fibrin is said not to be reduced even when there is anemia. In general, higher figures are obtained than normal, especially in the later stages, but not so high as in pneumonia. With cachexia the fibrin may fall with the other blood constituents. After hemorrhages in tuberculosis regeneration of the hemoglobin is very slow. In frank pulmonary tuberculosis the average hemoglobin is from 63 to 73 per cent. De Ruyter found no methemoglobin. Practically all reports agree in showing the low hemoglobin content, which is especially striking in the first stage.

<sup>6</sup> Myers and Short (*Jour. Biol. Chem.*, 1921 (48), 83), in their review on the potassium content of the blood state that the normal human serum contains rather less than 20 mg. per 100 cc., but they or the authors quoted by them give no figures in tuberculosis.

<sup>7</sup> Halverson, *Amer. Jour. Med. Sci.*, 1917 (68), 1309.

<sup>8</sup> Boenheim, *Beitr. Klin. Tuberk.*, 1921 (49), 233.

Numerous tables are given showing the protein content of the serum, which seems to vary with the amount of the anemia, wide variations being found. In the second stage concentration of the blood causes an increase in the percentage of total solids in the serum.

In 1905 Erben,<sup>9</sup> who has conducted many studies on the chemical composition of the blood in disease, reported his analyses in three cases of pulmonary tuberculosis. The first case was mild, ending in apparent recovery. The second case was one of pulmonary and regional tuberculosis moderately advanced, and the third, one of advanced tuberculosis near death. He gives an extended review of the literature on the composition of blood in tuberculosis and in other diseases, and summarizes the results of the analyses of all three of his cases as follows:

The total protein and the hemoglobin follow in quantity the red cell count. In the milder cases there was little difference from normal, but they were very low in the third case.

Fibrin was present in normal amounts in the two milder cases, but was doubled in the severe case. This fibrin increase in the late stage of tuberculosis has been observed by others.

Fat, lecithin and cholesterol were in normal quantities in the milder case and increased in the two more severe cases.

The quantity of ash was increased in the two more severe cases. Potassium and iron were decreased in the third case with the anemia, a condition which has been observed by others in advanced tuberculosis. Phosphoric acid was not much altered. Sodium and chlorine were increased in the more advanced cases. The quantity of calcium was progressively increased, while magnesium was decreased in the late stages.

Since Erben's studies there seem to be no reports of systematic chemical study of the blood in tuberculosis, but, with the development of modern methods of blood analyses, reports on the application of these methods have often included figures obtained in cases of tuberculosis. These may be discussed best, therefore, in connection with each blood constituent determined.

<sup>9</sup> Zeit. für Heilkunde, 1905 (26), 245.

## BLOOD LIPINS

*Fats and fatty acids.* We can find little specific discussion of the amounts of neutral fats and fatty acids in the blood in tuberculosis. The normal blood shows before breakfast about 0.57 to 0.82 per cent of ether soluble material in the plasma, of which but 0.1 to 0.2 per cent is neutral fat,<sup>10</sup> and this may be entirely absent from the corpuscles. Bloor finds some increase in total fatty acids in many diseases, including those associated with anemia, and hence high figures may be expected in tuberculosis but he includes no cases among his analyses.

The unsaturated fatty acids of blood are said to be generally increased in pathological conditions (Csonka)<sup>11</sup> but this author seems to have examined no cases of tuberculosis. The extensive studies on blood lipins by Feigl<sup>12</sup> also seem to have omitted tuberculous material. In their elaborate study of the chemistry of blood and tissues in disease, Dennstedt and Rumpf,<sup>13</sup> include but one case of tuberculosis (lungs, larynx and bowel), reporting a low fat content in the blood. With the above meager data may be included Erben's finding of normal total lipins in one early case and increased lipins in two advanced cases. Also the statement of Muggia<sup>14</sup> that the total fat of the blood is increased in advanced tuberculosis, which he suggests is related to the prevalence of fat deposition in the liver.

Henning<sup>15</sup> has studied the blood in 21 advanced cases of pulmonary tuberculosis and found fatty acids in amounts not far from those in normal blood. After accounting for the fatty acid present combined with lecithin and cholesterol, the residual amount is relatively high and since there was no visible lipemia the fatty acids could hardly be present as fat, so that some other form of combination is suggested. The ratios,  $\frac{\text{lecithin}}{\text{cholesterol}}$  and  $\frac{\text{cholesterol}}{\text{fatty acid}}$  are fairly constant for the entire series. They do not vary widely from those given for normal individuals.

<sup>10</sup> Bloor, Jour. Biol. Chem., 1916 (25), 577.

<sup>11</sup> Jour. Biol. Chem., 1918 (33), 401.

<sup>12</sup> Biochem. Zeit., Vols. 88 *et seq.*

<sup>13</sup> Zeit. klin. Med., 1905 (58), 84.

<sup>14</sup> Riforma Medica, 1922 (38), 73.

<sup>15</sup> Jour. Biol. Chem., 1922 (53), 167.

*Cholesterol.*<sup>16</sup> The cholesterol content of the whole blood is normally about 0.14 to 0.17 per cent (140 to 170 mgm. per 100 cc.) and it is about the same in the plasma, although it tends to be a trifle higher in the plasma and to show here greater variations in disease than in the corpuscles. It parallels closely the total fatty acids except in severe lipemia, and occurs both free and as cholesterol esters. According to Bloor and Knudson<sup>17</sup> about 33.5 per cent of the cholesterol of whole blood is combined as esters, and in the plasma 58 per cent is thus bound. In general, high figures for blood cholesterol have been reported in arteriosclerosis, nephritis, diabetes, obstructive jaundice, pregnancy and certain skin diseases, with low figures in anemia, but Denis<sup>18</sup> found hypercholesterolemia only in diabetes, while low figures were invariably found in conditions where marked prostration or cachexia exist. Unfortunately Denis gives no figures on the blood in tuberculosis.

As to findings in tuberculosis, Bacmeister and Henes<sup>19</sup> state that while with acute infections the blood cholesterol falls as the temperature rises, in tuberculosis the general condition of the patient seems to be the most important factor, for even with high fever normal or even high cholesterol values may be obtained if the patient is in good condition, low figures being found in cachexia even with fever. Henes<sup>20</sup> gives the following figures in 6 cases of pulmonary tuberculosis in milligrams per 100 cc. serum: 89, 97, 120, 150, 159, 195. Of these the first 2 were more advanced than the last 4. Chauffard *et al*<sup>21</sup> give analyses of 6 cases without fever and in good condition, with serum cholesterol ranging from 130 to 180 mgm., whereas in eight advanced cases with fever the figures ranged from 40 to 110 mgm. Weltman<sup>22</sup> reports that in early tuberculosis normal cholesterol figures may be obtained, but in advanced cases the cholesterol is low, and it falls rapidly in acute active cases, so that he ascribed to the cholesterol figure

<sup>16</sup> Review on the blood cholesterol is given by V. C. Myers, *Jour. Lab. Clin. Med.*, 1920 (5), 776.

<sup>17</sup> *Jour. Biol. Chem.*, 1917 (29), 7.

<sup>18</sup> *Jour. Biol. Chem.*, 1917 (29), 93.

<sup>19</sup> *Deut. med. Woch.*, 1913 (39), 544.

<sup>20</sup> *Deut. Arch. klin. Med.*, 1913 (111), 122.

<sup>21</sup> *Compt. Rend. Soc. Biol.* Quoted by Henes.

<sup>22</sup> *Wien. klin. Woch.*, 1913 (26), 874.

a prognostic significance in tuberculosis. By a crude comparative method v. Eisler and Laub<sup>23</sup> also found evidence of a cholesterol decrease progressing with the advance of the disease, and measured by the capacity of the free cholesterol to inhibit saponin hemolysis the decrease concerns chiefly the cholesterol esters. On the other hand, Neilson and Wheelon<sup>24</sup> found a slight but distinct increase in resistance to saponin hemolysis in tuberculosis, which indicates increased blood cholesterol, but the stage of the disease was not taken into consideration. Rosenthal and Petrzak,<sup>25</sup> who found that in war malnutrition the serum cholesterol was usually low, obtained in 12 of 14 cases of tuberculosis, serum cholesterol figures ranging from 50 to 85 mgm. per 100 cc. They attribute to the low blood lipoid content of malnutrition a factor in the decreased resistance to tuberculosis. Hence we find that there is general agreement that with advanced tuberculosis there is a marked decrease in the cholesterol content of the serum, but in early cases normal or even high figures may be obtained.

A more recent communication by Henning<sup>15</sup> indicates that cholesterol was found by the non-saponification method to be practically normal, but the saponification method gave results uniformly much lower (averaging about 50 per cent). The difference between the values for cholesterol by the two methods is very striking and since ordinary cholesterol is not appreciably affected by saponification, the presence of some substance other than cholesterol which gives the cholesterol color reaction but which is sensitive to alkali, is indicated. Since the value found without saponification is the same as similar values for normal blood it appears that in tuberculosis true cholesterol is replaced in considerable part by the unknown substance, which may, however, be closely related to cholesterol, since it gives the same color reaction. Henning states that the reason for the apparent substitution for cholesterol in tuberculosis blood is at present a matter of speculation, but when the lipoid nature of the tubercle bacillus and of the tubercles is considered, it seems reasonable to suspect that cholesterol may be involved in some way in the attempt of the body to protect itself from this organism.

<sup>23</sup> Wien. klin. Woch., 1913 (26), 968.

<sup>24</sup> Jour. Lab. Clin. Med., 1921 (6), 487.

<sup>25</sup> Berl. klin. Woch., 1919 (56), 793.

*Lecithin*, which is normally present in the plasma,<sup>26</sup> 0.20 to 0.24 per cent, in the corpuscles 0.38 to 0.42 per cent, and in the whole blood about 0.30 per cent (Bloor) is commonly found decreased in diseases associated with anemia or cachexia, and therefore presumably in tuberculosis, especially as it is increased in few or no pathological conditions. Calmette,<sup>27</sup> however, believed that free lecithin, at least, is increased in the serum in tuberculosis, for in tuberculosis the serum exhibits the power to activate the hemolytic agent of cobra venom. This fact, of itself alone, however, is not adequate proof that there is an actual increase in free lecithin, since conceivably there may be other explanations for the activation of cobra venom. No definite figures for blood lecithin in tuberculosis have been found, except those of Henning,<sup>15</sup> who in 21 cases of advanced tuberculosis found in the plasma from 0.194 to 0.245 per cent, or quite within the normal range.

#### BLOOD PROTEINS

As mentioned in the discussion of the early work on blood chemistry in tuberculosis, Erben found a slight relative increase in the globulin fraction. As far back as 1854, Becquerel and Rodier had recorded that the total protein content was decreased in tuberculosis, among other diseases. More recent investigators have done much work on the changes in the blood proteins, especially on the ratio of albumin to globulin, the latter usually being found increased in disease and during artificial immunization.<sup>28</sup>

Epstein<sup>29</sup> is quoted as finding globulin normal or decreased in tuberculosis but he mentions only one case of "influenzal or tuberculous pneumonia." In his extensive studies by newer methods Rowe mentions only one case of tuberculosis of the pleura with a slightly increased globulin fraction (38 per cent, normal average being 25 per cent). Hurwitz and Meyer<sup>30</sup> found that a rabbit

<sup>26</sup> Feigl gives 0.25 to 0.27 per cent as the normal lecithin content of the plasma (Biochem. Zeit., 1918 (90), 361).

<sup>27</sup> Berl. klin. Woch., 1914 (51), 496.

<sup>28</sup> Literature by Rowe, Arch. Int. Med., 1916 (18), 455.

<sup>29</sup> Jour. Exp. Med., 1912 (16), 719.

<sup>30</sup> Jour. Exp. Med., 1916 (24), 515.

inoculated intravenously with bovine tubercle bacilli exhibited a marked change in the globulin-albumin ratio of the blood, the globulin rising from 1.3 to 4.5 per cent, the albumin falling from 5.3 to 0.5 per cent, the A:G ratio changing from 80:20 to 10:90; at the same time the total protein fell from 6.6 to 5.0 per cent.

Frisch<sup>31</sup> corroborates Nast and Alder in finding generally an increase in the serum proteins in advanced phthisis, although, if cachexia is marked, the figures may be subnormal. Alder<sup>32</sup> found that the proportion of albumin to globulin, which is normally 65-80 : 35-20, alters in the direction of an increase in the globulin in direct proportion to the activity of the pulmonary lesions; in severe cases the proportion may be completely reversed, even in terminal stages with low total protein figures. Fibrotic and glandular tuberculosis have no effect on the albumin-globulin ratio. Peters<sup>33</sup> corroborated the occurrence of an increase in globulin in tuberculosis but could not correlate this change with the form or degree of lesion in any useful way, beyond the finding that if an increased albumin and globulin content falls the prognosis is better than if only the globulin decreases while the albumin remains high. If both albumin and globulin are high the prognosis is bad.

In tuberculous children, especially, high protein content is often found in the plasma (Mensi),<sup>34</sup> rising parallel with the tuberculin reaction, frequently falling shortly before death when the tuberculin reaction disappears.

In his extensive discussion of the quantitative relations of the blood proteins as revealed by the refractometric method, Reiss<sup>35</sup> gives no figures for tuberculosis but finds that the protein is decreased in cachexia and says that "quite similar relations are found in cancer, phthisis, etc." Peters<sup>36</sup> also found a slight diminution in the total serum protein in pulmonary tuberculosis, which is not altered by residence in high altitudes.

<sup>31</sup> Beitr. Klin. Tuberk., 1921 (48), 145.

<sup>32</sup> Zeit. f. Tuberk., 1920 (31), 10.

<sup>33</sup> Zeit. f. Tuberk., 1921 (35), 196.

<sup>34</sup> Pediatria, 1921 (29), 577.

<sup>35</sup> Ergeb. inn. Med. u. Kinderheilk., 1913 (10), 531.

<sup>36</sup> Zeit. physik. diätet. Therap., 1921 (25), 548.

Meyer-Bisch<sup>37</sup> in 10 cases of pulmonary tuberculosis in the second and third stages, found the concentration of serum proteins generally normal (7 to 8 per cent), although averaging somewhat above normal and in some severe cases high protein concentration (9.1 to 9.2 per cent) were present; the lowest figures were obtained in the cases with cachexia. The red corpuscles, however, were usually poor in protein with a correspondingly low hemoglobin figure, but not greatly changed even in cases with severe cachexia.

Gram<sup>38</sup> has studied the fibrinogen content of the blood plasma in many diseases. His normal figures varied between 0.20 and 0.38 per cent, with mean values of 0.27 per cent in men and 0.29 per cent in women. Acute infectious diseases usually produce a rise in the fibrinogen figure, pneumonia leading (average figure, 1.05 per cent) as has long been known. Closed tuberculosis showed figures not much above normal (average, 0.36 per cent in 7 cases, variations from 0.43 to 0.25 per cent) but in active tuberculosis much higher figures were obtained (average, 0.62 per cent in 22 cases, variations from 1.22 to 0.33 per cent), those complicated with acute coccus lesions naturally showing high figures.

Using the refractometric method, Frisch<sup>31</sup> also found that nodular and pneumonic forms of pulmonary tuberculosis are associated with a much increased fibrinogen figure in the plasma, as also is the case in intestinal and serous membrane tuberculosis; in fibrotic pulmonary and in caseous glandular tuberculosis there is a moderate increase, while latent pulmonary or dry pleural tuberculosis shows little or no increase in fibrinogen.

Frisch and Starlinger<sup>39</sup> estimate the fibrinogen content of the blood by mixing 0.2 cc. of a 5 per cent solution of sodium citrate with 0.8 cc. of blood, and centrifuge or wait for spontaneous sedimentation. Then 0.2 cc. of the citrated plasma is mixed with the same amount of a saturated sodium chlorid solution, and the flocculation that ensues in the next three minutes is recorded. In normal conditions a fibrinogen content of 0.2 per cent is the rule. When 0.4 or more is found, and pregnancy and menstruation can be excluded, then something is wrong in the organism.

<sup>37</sup> Zeit. exp. Path. u. Ther., 1919 (20), 54.

<sup>38</sup> Arch. Int. Med., 1921 (28), 312.

<sup>39</sup> Med. Klin., 1922 (18), 247; Zeit. ges. exp. Med., 1921 (24), 142.

If other signs point to tuberculosis, this intense flocculation is corroboratory evidence. It throws light on the activity and progressive nature of the tuberculous process, and is especially instructive when supplemented with the suspension stability test of the erythrocytes, which is likewise conditioned by the fibrinogen content of the blood. This, in turn, is the gage of the cell destruction going on, measured by the corresponding amount of albumin disintegration. No constant relation was found between the tuberculin reaction and the fibrinogen content. Exposure of the tuberculous lungs to x-rays may cause a transitory rise in blood fibrinogen.

Erik Wolff<sup>40</sup> states that albumose-like material is often present in the blood in tuberculosis. This is based on the principle that a higher residual nitrogen figure with metaphosphoric acid than with phosphomolybdic acid depends on the presence of albumose or similar substances, and in 3 of 6 cases of tuberculosis he found increased "albumose" nitrogen (10 to 15.5 mgm., normal being 1 to 7.5 mgm.) together with albumosuria. Hahn<sup>41</sup> found a similar evidence of increased amounts of higher products of protein cleavage in a small number of cases of tuberculosis.

#### NON-PROTEIN CONSTITUENTS

Despite the large amount of work that has been done on the chemistry of the non-protein nitrogenous constituents of the blood, little systematic investigation of any possible changes in these components in tuberculosis seems to have been made, presumably because it is assumed that such changes will be observed only when there is renal damage.

Schwarz and McGill<sup>42</sup> in their extensive study of the blood chemistry in many diseases, note that 2 of 6 cases of pulmonary tuberculosis showed an increased blood urea, one being an acute febrile case of miliary tuberculosis with toxic nephritis and nearly complete suppression of phthalein excretion, and in the other there was a mild parenchymatous nephritis. Tileston and Com-

<sup>40</sup> Inaug. Dissertation, Lund, Sweden, 1920.

<sup>41</sup> Biochem. Zeit., 1921 (121), 264.

<sup>42</sup> Arch. Int. Med., 1916 (17), 42.

fort<sup>43</sup> had previously found a slight retention in 2 of 3 cases, all 3 showing casts and traces of albumin in the urine. Kast and Wardell<sup>44</sup> also included a few cases of tuberculosis in their series, with urea nitrogen from 12 to 13 mgm. per 100 cc. in most of them, 2 only being as high as 18 to 20 mgm.

In a large number of children suffering from various diseases, Chapin and Myers<sup>45</sup> made blood analyses, and among them were several cases of tuberculosis of the various forms seen in children, but the figures for urea, creatinine and sugar were always quite within normal range. A few cases included in another study by Rosenberg<sup>46</sup> showed normal blood figures for urea, creatinine and indican.

### *Sugar*

Seitz<sup>47</sup> reports that persons with surgical tuberculosis, like subjects of staphylococcus infections, commonly exhibit a tendency to develop dietary hyperglycemia. Langston,<sup>48</sup> in a study of the blood sugar changes after giving 100 grams of glucose on an empty stomach, found that 60 per cent of cases of tuberculosis and 81 per cent of cases of carcinoma, showed a similar type of hyperglycemia. In these cases the rise of the blood sugar is higher, to 200 mgm., and the fall to normal slower than in normal persons. Similar curves are given in diabetes and hyperthyroidism. The reason for this alimentary hyperglycemia in tuberculosis has not been ascertained. McBrayer<sup>49</sup> found that in about one-third of his cases of chronic pulmonary tuberculosis there is an increase in the blood sugar accompanying a rise in basal metabolism rate of over 10 per cent; in about one-fifth of all such cases there may be an increased metabolic rate with normal blood sugar, or just the reverse. Rarely are both decreased or one normal and one decreased.

<sup>43</sup> Ibid., 1914 (14), 620.

<sup>44</sup> Ibid., 1918 (22), 581.

<sup>45</sup> Amer. Jour. Dis. Chil., 1919 (18), 555.

<sup>46</sup> Berl. klin. Woch., 1916 (53), 1314.

<sup>47</sup> Mitteil. Grenz Med. u. Chir., 1922 (34), 514.

<sup>48</sup> Jour. Lab. Clin. Med., 1922 (7), 293.

<sup>49</sup> Jour. Amer. Med. Assoc., 1921 (77), 861.

*Bilirubin*

In a study of the bilirubin content of the blood in various diseases, Bauer and Spiegel<sup>50</sup> included 3 cases of tuberculosis, all of which gave high bilirubin figures, but this small number of cases is of little significance.

*Blood gases*

As Lippert<sup>51</sup> found that reduction in the respiratory area caused a fall in the O and an increase in the CO<sub>2</sub> content of the arterial blood, it is to be presumed that similar changes occur whenever much lung tissue is involved by tuberculosis, or when the lung is collapsed by pleural effusions, but we have found no reports of direct blood gas analyses in tuberculosis.

*Chlorides*

More recent observations are not in agreement with the findings of the earlier investigators, for they agree that the blood chlorides are strikingly low in tuberculosis.

Boenheim<sup>52</sup> found the proportion of NaCl to decrease in direct proportion to the severity of the condition, and Bönninger<sup>53</sup> considers the hypochloremia to be the result of the tuberculosis itself and independent of the fever. As a result of this there may be a deficient secretion of HCl in the gastric juice. Boenheim has studied the chloride metabolism in tuberculosis and believes that there is an actual dechloridization of the body through excessive renal elimination. The tissues may become so depleted in advanced cases that a considerable part of the chloride given by mouth may be retained in the body.

In view of the reputed importance of renal changes in tuberculosis (see p. 318) a thorough study of the blood chemistry is desirable.

<sup>50</sup> Deut. Arch. klin. Med., 1919 (12), 17.

<sup>51</sup> Beitr. Klin. Tuberk., 1912 (24), 390. See also Heuer and Andrus, Bull. Johns Hopkins Hosp., 1922 (33), 130.

<sup>52</sup> Beitr. Klin. Tuberk., 1921 (49), 233.

<sup>53</sup> Zeit. exp. Path. u. Ther., 1919 (20), 63.

## ALKALI RESERVE IN THE BLOOD IN TUBERCULOSIS

Whitney<sup>54</sup> has called attention to the very severe degrees of acidosis that may occur in the terminal stages of disease of whatever sort, often sufficient apparently to account for the death or at least to add seriously to the existing intoxication. Unfortunately, in the cases examined by him were no typical examples of advanced pulmonary tuberculosis, but since anemia, terminal infections and cardiac decompensation commonly exhibit marked terminal acidosis, it is to be expected that a final acidosis is present in phthisis. The numerous studies on the subject of acidosis in various diseases,<sup>55</sup> also omit consideration of tuberculosis, except for inclusion of isolated cases which as such are of no significance. Children with tuberculosis, as well as those with many other diseases, often exhibit acetonuria,<sup>56</sup> but this is not usually associated with acidosis. Hemorrhage causes a transitory fall in the alkali reserve, but this is quickly compensated or overcompensated.<sup>57</sup>

Hachen<sup>58</sup> has made a study of the alkali reserve in 213 tuberculous patients having every variety of lesion, the far advanced type predominating, and states that a change for the worse in the clinical condition was always accompanied by a mild corresponding drop in the alkali reserve, but as a rule no depletion in the blood alkali reserve is seen until after the lesion has become far advanced and is accompanied by rather severe clinical symptoms. The blood alkali reserve in an individual case continues to decrease slowly with approaching death until a minimum of 50 is reached. The blood alkali reserve was 3 points lower in cases where the urine as voided was neutral or acid to methyl red. An acid urine is, of course, not an indication that an "acidosis" exists. An increase in temperature above 100°F. was usually accompanied by a slight decrease in the alkali reserve. There was no correlation between the respiratory or pulse rate and the blood alkali reserve. The alkali reserve was comparatively low (52) in two

<sup>54</sup> Arch. Int. Med., 1917 (20), 931.

<sup>55</sup> Review by Frothingham, Arch. Int. Med., 1916 (18), 717.

<sup>56</sup> Garland, Arch. Pediatrics, 1919 (36), 468.

<sup>57</sup> Evans, Brit. Jour. Exp. Path., 1921 (2), 105.

<sup>58</sup> Arch. Int. Med., 1922 (29), 705.

cases of acute miliary tuberculosis. Urochromogen or diazo substance in urine was frequently found when the blood alkali reserve was relatively low. In 13 cases showing at necropsy extensive tuberculous involvement of all lobes, the average alkali reserve was 56. Although there is a decrease in the alkali reserve as the case advances, at no time is there a marked "acidosis" in pulmonary tuberculosis.

#### ENZYMES OF THE BLOOD IN TUBERCULOSIS

##### *Proteolysis*

The normal blood serum contains an agent, presumably a peptolytic enzyme, which splits glycyl-tryptophane in small amounts, but in advanced tuberculosis, as well as in other conditions in which cellular disintegration liberates the enzyme, the amount is considerably increased (Mandelbaum<sup>59</sup>). After death the amount increases rapidly, especially in tuberculosis. Guggenheim<sup>60</sup> has found that in some diseases the blood serum has the capacity to augment tissue autolysis (as measured by the production of incoagulable nitrogen by autolyzing brain tissue), but in tuberculosis the effects were irregular (1 positive, 3 negative). Connio<sup>61</sup> states that the serum in tuberculosis has less inhibiting effect on the autolysis of tissues than normal serum, but this statement has apparently not been confirmed.

The capacity of the blood to digest Witte peptone was found to be very slightly above normal in the 2 cases of phthisis included in Saxl's<sup>62</sup> series of observations.

Grönberg<sup>63</sup> reports that in pulmonary tuberculosis the serum has a heightened capacity to digest lung tissue (Abderhalden test), but Gumpertz<sup>64</sup> found only a slight degree of specificity with various tuberculous organs as antigen, obtaining reactions not infrequently with substrates of tissues not involved in the case from which the serum was derived, as well as many negative

<sup>59</sup> Münch. med. Woch., 1914 (61), 461.

<sup>60</sup> Deut. med. Woch., 1914 (40), 63.

<sup>61</sup> Ann. inst. Maragliano, Vol. 5, p. 60.

<sup>62</sup> Berl. klin. Woch., 1914 (51), 824.

<sup>63</sup> Finska Lak. Handl., 1920 (62), 599.

<sup>64</sup> Beitr. Klin. Tuberk., 1914 (30), 200.

reactions in positive cases. A much more favorable opinion of this reaction is given by Jost,<sup>65</sup> as he found that in all cases of active pulmonary tuberculosis the serum digests lung tissue substrate, (ninhydrin test) but with inactive pulmonary lesions a negative result is usually obtained. Other lung diseases (carcinoma, bronchiectasis) may give positive reactions, which are never obtained when there is no pulmonary disease. If the liver and kidney are affected positive reactions may be obtained with these tissues as substrate, in which case the prognosis is unfavorable. Oeri<sup>66</sup> also reports a marked specificity in the hydrolysis of lung tissue by the serum of patients with pulmonary tuberculosis (38 of 40 positive), and others report equally specific results in bovine tuberculosis.<sup>67</sup>

#### *Fat-splitting enzymes (lipases)*

The literature on this subject is very confused because so many authors have failed to recognize that the capacity of a fluid to cause cleavage of a simple ester, like ethyl butyrate (*esterase*), may have no relation to its capacity to split natural fats (*lipase*). As a matter of fact, the technical difficulties are so great in the quantitative study of fat cleavage that virtually all the work reported as on lipase activity really concerns esterases splitting either ethyl butyrate, triacetin or tributyrin. Esterase has been found increased in the serum in early tuberculosis and decreased in advanced tuberculosis by Bauer.<sup>68</sup> A decrease in the power of the blood serum to split tributyrin was found by Caro,<sup>69</sup> to be common to cachexias from whatever cause, and Pincussohn<sup>70</sup> states that he has found a similar decrease in "some cases" of tuberculosis. A more extended study by Caro,<sup>71</sup> however, showed that in 8 of 9 early cases of tuberculosis there was a considerable increase in the tributyrin splitting activity of the serum, in second

<sup>65</sup> Beitr. Klin. Tuberk., 1919 (41), 125.

<sup>66</sup> Beitr. Klin. Tuberk., 1915 (35), 63.

<sup>67</sup> Hirsch and Mayer-Pullmann, Fermentforschung, 1920 (4), 64.

<sup>68</sup> Wien. klin. Woch., 1912 (25), 1376.

<sup>69</sup> Zeit. klin. Med., 1913 (78), 286.

<sup>70</sup> Deut. med. Woch., 1914 (40), 425.

<sup>71</sup> Zeit. klin. Med., 1920 (89), 49.

stage cases the results were irregular, in late cases with cachexia it was decreased, and in general there was no relation to the lymphocytosis of the disease.

Kotschneff<sup>72</sup> determined the enzymatic activity of the serum of guinea pigs and rabbits after the intraperitoneal injection of non-lethal doses of tubercle bacilli. She found a decrease in the lipase (monobutyryl esterase), an increase in the antitrypsin and nuclease, a slight decrease in amylase and diastase, and in guinea pigs a decrease in catalase.

Clerc<sup>73</sup> found a decrease in both lipase and amylase activity of the serum in tuberculosis, not only in man but in dogs and rabbits, which was very marked in severe cases. Carrière<sup>74</sup> corroborated this decrease in lipolytic (esterase) activity in human serum of advanced tuberculosis and reported an increase to follow treatment with sodium cacodylate. Garnier<sup>75</sup> found an increase in serum lipase (esterase) in cases of acute tuberculosis in relatively sound subjects, with a fall in chronic cases and a rise in cases showing improvement.

Kollert and Frisch<sup>76</sup> found tributyrin splitting more active with the serum from cases of tuberculosis with a favorable prognosis than in rapid, progressive tuberculosis or in cases with pleural exudates and hence believe that the lipase is in some way related to the immunity. "The more a tuberculosis is fibrous and stationary, the higher is the lipase content of the serum, and the more wasting the process or the more it tends to caseation, the lower the lipase figure." In pseudochylia the serum lipase was not found altered. They found no relation between the lipase figure and the lymphocyte count of the blood, agreeing with Caro. Shortly before death there is a marked fall in the serum lipase, and guinea pigs infected with tubercle bacilli show a similar fall.

Pribram<sup>77</sup> found a decrease in true lipase in the blood in 1 case of miliary tuberculosis, as determined by the production of acid when incubated with olive oil.

<sup>72</sup> Biochem. Zeit., 1913 (55), 481.

<sup>73</sup> Quoted by Kotschneff—loc. cit.

<sup>74</sup> Compt. Rend. Soc. Biol., 1899 (51), 989.

<sup>75</sup> Compt. Rend. Soc. Biol., 1903 (55), 1423.

<sup>76</sup> Beitr. Klin. Tuberk., 1920 (43), 305; 1921 (47), 146; 1921 (48), 15.

<sup>77</sup> Cent. inn. Med., 1908 (29), 81.

*Catalase*

Catalase activity of the blood is reduced in tuberculosis. Jolles and Oppenheim<sup>78</sup> found in 7 cases 2 with normal catalase, but five with marked reduction, the low results accompanying anemia. Robin and Fiessinger<sup>79</sup> also reported that 2 cmm. normal blood liberated 30 to 45 cc. gas, while the blood in tuberculosis liberated but 10 to 20 cc., and in cancer usually from 20 to 30 cc. Kotschneff<sup>72</sup> also found a decrease in blood catalase in tuberculous guinea pigs.

*Diastase*

Diastase is found in the blood, coming chiefly if not solely from the pancreas.<sup>80</sup> Although there have been numerous studies of the diastatic or amylase activity of the blood in disease, little attention has been given to tuberculosis. The review of the literature on amylase in the blood and urine by Stocks<sup>81</sup> makes no mention of tuberculosis. The only reference we have found is an article by Achard and Clerc<sup>82</sup> who state that they found normal amylase activity in the blood in incipient tuberculosis, decreased in febrile tuberculosis, and very much decreased in advanced phthisis; in general, very low figures presage death, not only in tuberculosis but in other diseases, thus paralleling the changes in the blood lipase. Kotschneff<sup>72</sup> found decrease in diastase in guinea pigs and rabbits infected with tubercle bacilli.

According to Kabuzoe<sup>83</sup> there is a marked decrease in the maltase content of the blood and viscera of tuberculous animals.

*Nuclease*

Nuclease, as determined by the optical method, is said by Wolter<sup>84</sup> to be increased in the serum in both human and guinea

<sup>78</sup> Virchow's Arch., 1905 (180), 185.

<sup>79</sup> Compt. Rend. Soc. Biol., 1910 (69), 414.

<sup>80</sup> Davis and Ross, Amer. Jour. Physiol., 1921 (56), 22; Block, Zeit. klin. Med., 1922 (93), 381.

<sup>81</sup> Quart. Jour. Med., 1916 (9), 216.

<sup>82</sup> Compt. Rend. Soc. Biol., 1901 (53), 708.

<sup>83</sup> Japan Med. World, 1921 (1), 19.

<sup>84</sup> Dissert., St. Petersburg, 1913; abst. Cent. f. Biochem., 1913 (16), 271.

pig tuberculosis. Although there were marked individual variations, there was a general increase in nucleinolytic activity in early stages, increasing in the second and third stages; in carcinoma a still more marked increase was observed.

### *Antitryptic activity*

Antitryptic activity of the blood is increased in tuberculosis as in other diseases with cachexia, according to Brieger and Trebing,<sup>85</sup> who call the antitrypsin increase a reaction of cachexia. Other observers, however, have found that this increase is not a constant or characteristic feature of cachexia, and R. Weil<sup>86</sup> found low figures in some cases of advanced tuberculosis. Others<sup>87</sup> have found that in tuberculosis without mixed infection there is a high antitryptic titer, but that when there is a high leucocytosis the antitrypsin decreases. As a rule, however, there is an increased antitryptic titer in cachexia from whatever cause, but perhaps less constant and less severe in tuberculosis than in cancer and advanced exophthalmic goiter.<sup>88</sup>

### THE COAGULABILITY OF THE BLOOD

Coagulation time does not seem to be markedly altered in tuberculosis, except when shortened following large hemorrhages, despite the fact that there is usually more or less increase in the amount of fibrinogen in the blood in tuberculosis, varying directly with the acuteness of the process (Gram<sup>89</sup>). Rudolf and Cole<sup>90</sup> examined the blood in 9 cases of tuberculosis by a method which gave an average normal coagulation time of 7 minutes, and in these cases the average was 8.9 minutes, with extremes of 7 and 10.4 minutes. Addis<sup>90</sup> found normal coagulation time in 12 cases, Vierordt found the time shortened to 3 minutes in phthisis and to

<sup>85</sup> Berl. klin. Woch., 1908 (45), 1041, 1349, 2260.

<sup>86</sup> Arch. Int. Med., 1910 (5), 109.

<sup>87</sup> Wiens, Deut. Arch. klin. Med., 1907 (91), 456; Jochmann and Kantrowicz, Zeit. klin. Med., 1908 (66), 153.

<sup>88</sup> St. Rusznyák, Barát and Dániel, Wien. Arch. inn. Med., 1922 (3), 515.

<sup>89</sup> Amer. Jour. Med. Sci., 1911 (142), 481.

<sup>90</sup> Edinburgh Med. Jour. 1910 (5), 38.

5 minutes with cavity (normal  $9\frac{1}{2}$  minutes) while Solis-Cohen<sup>91</sup> found the following figures in 100 cases (normal by his method averaging  $8\frac{2}{3}$  minutes).

*Clotting-time in pulmonary tuberculosis*

|                                     | ALL STAGES      | FIRST STAGE    | SECOND STAGE   | THIRD STAGE     |
|-------------------------------------|-----------------|----------------|----------------|-----------------|
| Number of cases.....                | 100             | 26             | 27             | 47              |
| Number observations.....            | 134             | 32             | 32             | 70              |
| Average clotting-time, minutes..... | $5\frac{1}{6}$  | $5\frac{1}{7}$ | $5\frac{1}{7}$ | $5\frac{1}{5}$  |
| Maximum clotting-time, minutes..... | $10\frac{3}{8}$ | $8\frac{1}{2}$ | $8\frac{1}{2}$ | $10\frac{3}{8}$ |
| Minimum clotting-time, minutes..... | $2\frac{1}{8}$  | $2\frac{1}{8}$ | $2\frac{1}{2}$ | $2\frac{5}{8}$  |

The shortened coagulation-time may have been due to some factor other than the existence of tuberculosis. The patients were all drinking large quantities of milk which is said to hasten coagulation through the calcium it contains. Practically all the tests were made during the hot and humid summer months. In addition to the perspiration from this cause, some of the patients had the water content of their blood further depleted by night-sweats, while others perspired more than usual on account of cardiac weakness.

In 16 tuberculous women who were spitting blood, the time averaged  $5\frac{2}{3}$  to  $5\frac{3}{4}$  minutes irrespective of whether they were menstruating or not. Burns and Young<sup>92</sup> found that the degree of coagulability of the blood bore no relation to the probability of pulmonary hemorrhage, a result corroborated by Stähelin.<sup>93</sup> Surgical tuberculosis is accompanied by no deviation from normal coagulability (Nél).<sup>94</sup> Magnus-Alsleben<sup>95</sup> has called attention to the fact that the blood expectorated from pulmonary hemorrhages is more or less incoagulable, which he attributed to the presence of coagulation-inhibiting substances present in tuberculous lung tissue.

<sup>91</sup> Arch. Int. Med., 1911 (8), 684 and 824.

<sup>92</sup> Amer. Jour. Med. Sci., 1917 (154), 797.

<sup>93</sup> Beitr. Klin. Tuberk., 1919 (43), 28, bibliography.

<sup>94</sup> Inaug. Dis., Berlin, 1912. Quoted by Stähelin.

<sup>95</sup> Zeit. klin. Med., 1915 (81), 9.

## HEMOLYSIS IN TUBERCULOSIS

Chalier<sup>96</sup> found that the serum in tuberculosis does not contain autohemolysins, and rarely isohemolysins. With a normal number of erythrocytes, however, the hemoglobin index may be lowered. Despite this lack of evidence of marked hemolysis there is often intestinal siderosis which is usually considered the result of hemolysis. The red cells in tuberculosis may show an increased resistance to hemolysis by saponin, ascribed to an increase in blood cholesterol.<sup>24</sup>

Thompson<sup>97</sup> found that previous injection of tuberculin into rabbits increased their capacity to form hemolysins for foreign corpuscles, which he attributes to a stimulation of the endothelial phagocytes from which the antibodies may be derived.

## SEDIMENTATION OF CORPUSCLES

If the coagulation of the blood is prevented by oxalates, citrates or hirudin, the red cells show a tendency to agglutinate and fall out of the suspension in many pathological conditions, the speed of sedimentation depending on the degree of agglutination. In tuberculosis the speed of sedimentation is increased in direct proportion to the rate of progress of the disease, even in afebrile cases, according to Westergren.<sup>98</sup> This sedimentation is the result of the agglutination by the increased fibrinogen content of the plasma, as well as the decreased cell volume of the blood in tuberculosis.<sup>38</sup> It is sometimes increased with positive tuberculin reactions, but not in inactive tuberculosis. Schürer and Eimer<sup>99</sup> found it increased in all febrile diseases, whether acute or chronic, especially in rheumatism. Normal values tend to exclude active tuberculosis.<sup>100</sup> In tuberculosis with fever the speed of sedimentation varies from twice to nearly 10 times normal, and in afebrile cases an increased speed is of bad prognostic significance. In

<sup>96</sup> Jour. de Méd. de Lyon, 1920, p. 175.

<sup>97</sup> Jour. Med. Research., 1922 (43), 37.

<sup>98</sup> Acta. med. scandinav., 1921 (54), 247; Brit. Jour. Tuberc., 1921 (15), 72; Beitr. Klin. Tuberk., 1921 (46), 285.

<sup>99</sup> Berl. klin. Woch., 1921 (58), 1251.

<sup>100</sup> Katz, Zeit. f. Tuberk., 1922 (35), 401.

principle the sedimentation is merely a more accurate measurement of the phenomenon which in bygone times, as the *crusta phlogistica*, had a high reputation as indicating diagnosis and prognosis.

### *Stability of blood proteins*

Darányi<sup>101</sup> states that the serum of patients with much pathological tissue destruction is more readily precipitated by physical and chemical means (heat, phenol, alcohol, sublimate, etc.) than normal blood serum. In tuberculosis this reaction was found to be of value in estimating the activity of the process and especially the amount of tissue disintegration. Cases that recover show a disappearance of this abnormal precipitability.

### THE CHANGES IN THE BLOOD AFTER HEMORRHAGE

After severe hemorrhages the blood shows a decrease in specific gravity and viscosity, an increase in surface tension and electrical resistance, and either increase or decrease of the freezing-point depression, all these changes being transient if the individual is otherwise normal. In tuberculosis the return to normal will be slower in proportion to the progress of the disease. There is a rapid absorption of fluid from the tissues and tissue spaces, resulting in a dilution of protein and formed elements, but not of salts. For the same reason the density of the blood decreases in direct relation to the proportion of the total blood that has been lost.<sup>102</sup> The alkali reserve of the blood is somewhat lowered by severe hemorrhage,<sup>103</sup> but there is not a marked acidosis; this is quickly compensated or even overcompensated.<sup>104</sup> The total nitrogen of the blood of course falls, but there is a tendency for sugar (Tatum),<sup>103</sup> urea, amino-N<sup>105</sup> and non-protein N to increase, and there is increased elimination of creatine in the urine, presumably from destruction of muscle tissue to replace the lost blood proteins. Non-protein N increase is especially noted in febrile

<sup>101</sup> Abst. in Zent. f. Tuber., 1922 (17), 310.

<sup>102</sup> Richet *et al.*, Compt. Rend. Acad. Sci., 1918 (166), 587.

<sup>103</sup> Buell, Jour. Biol. Chem., 1919 (40), 29; Tatum, ibid., 1920 (41), 59.

<sup>104</sup> Evans, Brit. Jour. Exp. Path., 1921 (2), 105.

<sup>105</sup> György, and Zunz, Jour. Biol. Chem., 1915 (21), 511.

conditions,<sup>106</sup> the nitrogenous products of tissue destruction being washed from the tissues into the blood. There is said to be a decreased permeability of vessels, resulting in reduced exudative processes.<sup>107</sup> The proportion of the several blood proteins is variably altered after *repeated* hemorrhages; the sugar is little affected<sup>108</sup> but there may occur a marked rise in the content of immune bodies, especially specific agglutinins.<sup>109</sup> The serum anti-ferment is decreased.<sup>110</sup> When a definite anemia results from repeated small hemorrhages there may be a rise in both sugar and diastase.<sup>111</sup> Rapid large, or small repeated hemorrhages, cause a decrease in the coagulation time because of a decrease in anti-thrombin and a slight increase in prothrombin, in spite of a decrease in fibrinogen.<sup>112</sup> Lipemia is often produced by severe or repeated hemorrhages, with a great increase in the phospholipins and cholesterol of the plasma and corpuscles.<sup>113</sup> Feigl<sup>114</sup> found that the amount of lipemia following hemorrhages in pulmonary tuberculosis is not so high as is usually obtained with a corresponding loss of blood in sound persons.

Experimental studies have shown that regeneration of the blood proteins is most rapid on a protein-rich diet.<sup>115</sup> When an incomplete protein, such as gliadin, is the sole protein of the diet, the hemoglobin is not regenerated. Meat and hemoglobin-rich diets are especially favorable.<sup>116</sup>

<sup>106</sup> Lewy and Mendl, Deut. Arch. klin. Med., 1921 (136), 112.

<sup>107</sup> Luihlen, Med. Klin., 1913 (9), 1713.

<sup>108</sup> Taylor and Lewis, Jour. Biol. Chem., 1915 (22), 71.

<sup>109</sup> See Hahn and Langer, Zeit. Immunität., 1917 (26), 199.

<sup>110</sup> Petersen and Levinson, Jour. Amer. Med. Assoc., 1922 (78), 257.

<sup>111</sup> Karsner *et al.*, Jour. Exper. Med., 1921 (34), 349.

<sup>112</sup> Drinker, Amer. Jour. Physiol., 1915 (36), 305; Hanzlik, Jour. Lab. Clin. Med., 1920 (6), 59.

<sup>113</sup> Bloor and Farrington, Jour. Biol. Chem., 1920 (41), xlviii.

<sup>114</sup> Biochem. Zeit., 1921 (115), 63.

<sup>115</sup> Kerr *et al.*, Amer. Jour. Physiol., 1918 (47), 456.

<sup>116</sup> Amer. Jour. Physiol., 1920 (53), 151.

## CHAPTER IX

### CHEMISTRY OF TUBERCULOUS EFFUSIONS

#### THE COMPOSITION OF TUBERCULOUS EXUDATES OF PLEURA AND PERITONEUM

This usually falls between that of the simple non-inflammatory transudates and the acute inflammatory exudates that are produced by the pathogenic coccii. Even when produced by the same cause, effusions vary in composition in different parts of the body, presumably because of variations in the permeability of the vessels in different vascular areas; just as pleural, pericardial, peritoneal, and meningeal fluids normally differ from one another. Thus, C. S. Schmitt<sup>1</sup> found the composition of the transudates in different parts of the body of a patient who died of nephritis to have the following composition:

|                       | PLEURAL | PERI-TONEAL | SUBAR-ACHNOID | SUBCU-TANEOUS |
|-----------------------|---------|-------------|---------------|---------------|
| Water.....            | 963.95  | 978.91      | 983.54        | 988.70        |
| Solids.....           | 36.05   | 21.09       | 16.46         | 11.30         |
| Organic matter.....   | 28.50   | 11.32       | 7.98          | 3.60          |
| Inorganic matter..... | 7.55    | 9.77        | 8.48          | 7.70          |

The general rule is that while the proportion of salts remains nearly constant, the proportion of protein in edematous fluids in different localities varies in decreasing order as follows: (1) pleura; (2) peritoneum; (3) cerebrospinal; (4) subcutaneous.

An increase in solids occurs after the effusion has existed for some time, presumably because of absorption of water and salts, leaving a slowly increasing proportion of proteins. The composition of the patient's blood has considerable influence on the

<sup>1</sup> Hoppe-Seyler's Physiol. Chemie.

composition of the effusion; this is particularly true in the case of ascites from portal obstruction, the contents of the blood coming from the intestine during digestion modifying the composition of the ascitic fluid.<sup>2</sup> Thus Müller,<sup>3</sup> in a case of portal vein thrombosis, found in the ascitic fluid of a patient on an ordinary mixed diet, 0.179 per cent nitrogen; on a protein-rich diet, 0.2494 per cent N; on a protein-poor diet, 0.1764 per cent N. In cachectic conditions the proportion of proteins is less than in stronger individuals, and, as in the blood plasma, the albumin decreases more rapidly than the globulin as the cachexia advances (Umber).<sup>4</sup> All these various factors will, therefore, modify the composition of tuberculous exudates.

### *Physical chemistry of effusions*

The differences between transudates and exudates depend almost solely on their protein contents, for the non-protein elements are nearly identical with those of the lymph and blood plasma, which naturally must be so since any original or temporary deviation in osmotic pressure will be rapidly equalized by diffusion. Thus Bodon<sup>5</sup> found the concentration of the electrolytes nearly constant in spite of considerable differences in composition of various edema fluids, indicating that the serosa permits free passage of inorganic salts while holding back the organic substances. Transudates contain an excess of NaCl over other electrolytes, but in exudates the proportion of electrolytes other than chlorides is increased over the findings in transudates.<sup>6</sup>

Effusions are usually alkaline in the same sense as the plasma, except when bacterial changes lead to acid formation, but they are always able to neutralize less acid than the blood of the same individual (Opie).

<sup>2</sup> See Denis and Minot, Arch. Int. Med., 1917 (20), 879.

<sup>3</sup> Deut. Arch. klin. Med., 1903 (76), 563.

<sup>4</sup> Zeit. klin. Med., 1903 (48), 364.

<sup>5</sup> Pflüger's Arch., 1904 (104), 519.

<sup>6</sup> Gruner, Biochem. Jour., 1907 (2), 383.

The surface tension of exudates is lower than that of transudates,<sup>7</sup> depending chiefly upon the globulin content.

The molecular concentration of tuberculous and other exudates is usually lower than that of the blood (average  $\Delta$  of serum =  $0.56^\circ$ ). Achard and Loeper<sup>8</sup> found in 22 specimens of ascitic fluid the following depression of freezing point:

|   | $\Delta$                       |
|---|--------------------------------|
| 9 cases of cirrhosis.....               | $-0.49^\circ$ to $-0.54^\circ$ |
| 4 cases of tuberculous peritonitis..... | $-0.49^\circ$ to $-0.53^\circ$ |
| 5 cases of ovarian cyst.....            | $-0.52^\circ$ to $-0.58^\circ$ |
| 4 cases of abdominal cancer.....        | $-0.46^\circ$ to $-0.59^\circ$ |

Rzenthkowski<sup>9</sup> gives the following figures:

|   | $\Delta$                       | AVERAGE        |
|---|--------------------------------|----------------|
| 6 cases of tuberculous pleural effusions..... | $-0.50^\circ$ to $-0.53^\circ$ | $-0.523^\circ$ |
| 3 cases of tuberculous peritoneal effusions.. | $-0.52^\circ$ to $-0.53^\circ$ | $-0.523^\circ$ |
| 5 cases of cancerous peritoneal effusions.... | $-0.53^\circ$ to $-0.56^\circ$ | $-0.543^\circ$ |

Purulent exudates may show a high molecular concentration ( $-0.84^\circ$  in one case), due to decomposition of the proteins into crystalloids. In 2 cases of tuberculous ascites Cohn<sup>10</sup> obtained  $-0.501^\circ$  and  $-0.593^\circ$ .

Tieken<sup>11</sup> has found the results in transudates, exudates, and other body fluids shown in the following table:

<sup>7</sup> Trevisan, Zeit. exp. Path., 1911 (10), 141.

<sup>8</sup> Compt. Rend. Soc. Biol., 1901 (53), 621.

<sup>9</sup> Virch. Arch., 1905 (179), 405.

<sup>10</sup> Mitt. Grenz. Med. u. Chir., 1906 (15), 27.

<sup>11</sup> Amer. Medicine, 1905 (10), 822.

| NATURE OF FLUID          | SPECIFIC GRAVITY | FREEZING-POINT OF EFFUSION | FREEZING-POINT OF BLOOD | DISEASE                 |
|--------------------------|------------------|----------------------------|-------------------------|-------------------------|
| Pleuritic effusion.....  | 1,016            | -0.55                      | -0.56                   | Pneumonia, lobar        |
| Pleuritic effusion.....  | 1,018            | -0.55                      | -0.55                   | Pneumonia, lobar        |
| Pleuritic effusion.....  | 1,018            | -0.54                      | -0.56                   | Tuberculosis            |
| Pleuritic effusion.....  | 1,020            | -0.55                      | -0.56                   | Tuberculosis            |
| Pleuritic effusion.....  | 1,016            | -0.55                      | -0.56                   | Tuberculosis            |
| Pleuritic effusion.....  | 1,018            | -0.64                      | -0.56                   | Valvular heart disease  |
| Pleuritic effusion.....  | 1,030            | -0.60                      | -0.58                   | Empyema; cyanosis       |
| Pericardial effusion ... | 1,018            | -0.55                      | -0.56                   | Pericarditis            |
| Pericardial effusion ... | 1,016            | -0.56                      | -0.56                   | Pericarditis            |
| Pericardial effusion ... | 1,012            | -0.56                      | -0.56                   | Hydropericardium        |
| Ascitic fluid.....       | 1,024            | -0.60                      | -0.56                   | Cirrhosis of liver      |
| Ascitic fluid.....       | 1,020            | -0.57                      | -0.56                   | Cirrhosis of liver      |
| Ascitic fluid.....       | 1,018            | -0.58                      | -0.56                   | Tuberculous peritonitis |
| Ascitic fluid.....       | 1,013            | -0.62                      | -0.56                   | Organic heart disease   |
| Ascitic fluid.....       | 1,035            | -0.65                      | -0.58                   | General peritonitis     |
| Hydrocele fluid.....     | 1,016            | -0.56                      | -0.56                   | Tuberculosis            |
| Cerebrospinal fluid....  | 1,018            | -0.62                      | -0.58                   | Uremic coma             |
| Cerebrospinal fluid....  | 1,016            | -0.64                      | -0.68                   | Uremic coma             |
| Cerebrospinal fluid .... | 1,020            | -0.64                      | -0.64                   | Uremic coma             |
| Cerebrospinal fluid .... | 1,014            | -0.56                      | -0.56                   | Tuberculous meningitis  |
| Cerebrospinal fluid .... | 1,017            | -0.56                      | -0.56                   | Epidemic meningitis     |
| Cerebrospinal fluid .... |                  | -0.56                      | -0.56                   | Epidemic meningitis     |

### Protein contents

As indicated in the table given previously (p. 209), these vary greatly in quantity in various fluids<sup>12</sup> according to the cause of the edema and the place where it occurs. In general, non-inflammatory edemas (transudates) contain much less protein than do the inflammatory exudates, as is shown by the following table of analyses by Halliburton<sup>13</sup> and by Bernheim's<sup>14</sup> determinations of proteins in ascitic fluids.

<sup>12</sup> See also v. Jaksch, Zeit. klin. Med., 1893 (23), 225; Rzentkowski (*loc. cit.*).

<sup>13</sup> Adami, Allbutt's System, 1896 (1), 97.

<sup>14</sup> Quoted by Hammarsten, "Physiological Chemistry."

TABLE 1

|   | SP. GR. | PARTS PER 100 CC. OF FLUID |        |                |               |
|---|---------|----------------------------|--------|----------------|---------------|
|   |         | Total protein              | Fibrin | Serum-globulin | Serum-albumin |
| Acute pleurisy .....                        | 1.023   | 5.123                      | 0.016  | 3.002          | 2.114         |
| Acute pleurisy .....                        | 1.020   | 3.4371                     | 0.0171 | 1.2406         | 1.1895        |
| Acute pleurisy .....                        | 1.020   | 5.2018                     | 0.1088 | 1.76           | 3.330         |
| Hydrothorax. }<br>Average of 3 cases }..... | 1.014   | 1.7748                     | 0.0086 | 0.6137         | 1.1557        |

TABLE 2

| ASCITIC FLUID IN                       | PARTS OF PROTEIN TO 1000 CC. FLUID |       |            |
|--|------------------------------------|-------|------------|
|  | Max.                               | Min.  | Mean       |
| Cirrhosis of the liver.....            | 34.5                               | 5.6   | 9.69-21.06 |
| Bright's disease.....                  | 16.11                              | 10.10 | 15.6-10.36 |
| Tuberculous and idiopathic peritonitis | 55.8                               | 18.72 | 30.7-39.95 |
| Carcinomatous peritonitis.....         | 54.20                              | 27.00 | 35.1-58.96 |

As a rule tuberculous fluids are rich in proteins. Thus in 12 tuberculous pleural effusions Gloyne<sup>15</sup> found an average of 5.06 per cent of protein (extremes 2.5 and 6.75 per cent), total solids 7 per cent; ash, 0.63 per cent. Similar figures, however, are obtained in cancerous effusions.

The quantitative relations of the different varieties of proteins have been less studied. Serum-albumin and globulins constitute by far the largest part of the proteins, fibrinogen being scanty except in some inflammatory exudates, so that coagulation very seldom occurs spontaneously; of such spontaneously coagulating exudates the fluid in tuberculous meningitis is one of the best examples. The differences in the proportion of different serum proteins in effusions is attributed by A. Oswald<sup>16</sup> to the relative viscosity of these proteins, which determines their ability

<sup>15</sup> Lancet, 1913 (2), 1534.

<sup>16</sup> Zeit. exp. Path., 1910 (8), 226.

to pass through the capillary walls. The viscosity of serum proteins varies in the following increasing order: albumin, pseudoglobulin, euglobulin and fibrinogen; hence in transudates we may find only the first two, or perhaps only the albumin, while in exudates the two latter appear. Because of their exudative character we should expect tuberculous effusions to contain a relatively large amount of globulin and a little fibrinogen, which corresponds to the tendency for delicate fibrin masses to appear spontaneously in tuberculous exudates.

Joachim<sup>17</sup> found in pleural transudates and exudates that the proportion of albumin, euglobulin, and pseudoglobulin is always lower in hydrothorax than in pleurisy, including tuberculous pleuritis. Of different forms of ascites, the largest proportion of globulin and the smallest of albumin occur in cirrhosis, while with carcinoma the proportions are reversed, and in tuberculosis the proportions are extremely variable (see table, p. 215). In general the albumin is more abundant than the globulin,<sup>18</sup> but, as Umber<sup>4</sup> has found, the proportion of albumin sinks more rapidly in cachexia than does the globulin, corresponding to the similar changes in the blood proteins. The amount of protein lost in exudates is strikingly shown by one of Umber's cases of cancerous ascites; during one year the fluid removed by paracentesis contained not less than 3 kilos of pure protein, the patient weighing but 55.5 kilos. Of course such figures will not be obtained in tuberculous exudates, and yet there may be a noteworthy loss of protein from repeated aspirations of tuberculous pleural or peritoneal effusions.

Several authors have found in inflammatory ascitic exudates a protein having physical and chemical properties much resembling mucin; it has been especially studied by Umber,<sup>19</sup> who finds it quite similar to the synovial mucin isolated in arthritis by Salkowski, and calls it *serosamucin*. He found it present in tuberculous as well as in other sorts of effusions.

<sup>17</sup> Pflüger's Arch., 1903 (93), 558.

<sup>18</sup> See Epstein, Jour. Exp. Med., 1914 (20), 334.

<sup>19</sup> Zeit. klin. Med., 1903 (48), 364; also Holst, Upsalalakar, Forhand., 1904, p. 304.

The distribution of the different protein fractions in tuberculous effusions seems to be very variable, according to the results obtained by Joachim,<sup>17</sup> which are as follows:

*Proteins of tuberculous exudates (Joachim)*

| SPECIFIC GRAVITY | GRAMS N PER 100 CC. FLUID |                  |            |                 |         | RELATION OF TOTAL GLOBULIN TO ALBUMIN | EUGLOBULIN IN TOTAL GLOBULIN FRACTIONS<br>per cent |
|------------------|---------------------------|------------------|------------|-----------------|---------|---------------------------------------|--|
|                  | Total N                   | Total coagulable | Euglobulin | Pseudo-globulin | Albumin |                                       |  |
| Peritonitis..... | 1016                      | 0.497            | 0.422      | 0.0753          | 0.1579  | 0.1885                                | 55:44 32.3   |
| Peritonitis..... | 1021                      | 0.9065           | 0.8943     | 0.2037          | 0.1246  | 0.525                                 | 38:61 62   |
| Peritonitis..... | 1020                      | 0.8715           | 0.812      | 0.2541          | 0.1488  | 0.3924                                | 50:49 63   |
| Peritonitis..... | 1017                      | 0.5167           | 0.4988     | 0.1276          | 0.1842  | 0.1869                                | 62:37 40.9   |
| Pleuritis.....   | 1022                      | 0.816            | 0.7898     | 0.1598          | 0.21    | 0.42                                  | 47:53 42.6   |

The following is a comparison of the protein contents of tuberculous ascites fluid with that from other conditions, compiled from Joachim's figures, in per cent of total protein.

| DIAGNOSIS                | EUGLOBULIN | PSEUDO-GLOBULIN | ALBUMIN | TOTAL GLOBULIN | RELATION OF TOTAL GLOBULIN TO ALBUMIN |
|--------------------------|------------|-----------------|---------|----------------|---------------------------------------|
| Cirrhosis.....           | 25.56      | 37.29           | 37.15   | 62.85          | 1:0.59                                |
| Cardiac dropsy.....      | 20.43      | 27.39           | 52.18   | 47.82          | 1:1.1                                 |
| Cancerous ascites.....   | 12.44      | 22.63           | 64.92   | 35.07          | 1:1.8                                 |
| Tuberculous ascites..... | 17.83      | 37.41           | 44.76   | 55.24          | 1:0.8                                 |
| Tuberculous ascites..... | 23.87      | 14.6            | 61.53   | 38.47          | 1:1.6                                 |
| Tuberculous ascites..... | 31.95      | 18.71           | 49.34   | 50.12          | 1:1                                   |
| Tuberculous ascites..... | 25.59      | 36.93           | 37.47   | 62.52          | 1:0.6                                 |

As Joachim himself says, no regularity can be found in the proportion of the three proteins in tuberculous exudates.

We are indebted to Epstein<sup>18</sup> for analyses of pleural and peritoneal fluids in various conditions, his figures being as follows:

*Pleural effusions*

| CASE NUMBER                     | TOTAL PROTEIN              | INCOAGULABLE NITROGEN | TOTAL GLOBULIN | EUGLOBULIN | PSEUDOGLOBULIN | ALBUMIN | CHLORIDES | ASH   | RATIO OF GLOBULIN TO ALBUMIN | PER CENT OF GLOBULIN IN PROTEIN |
|---------------------------------|----------------------------|-----------------------|----------------|------------|----------------|---------|-----------|-------|------------------------------|---------------------------------|
|                                 | Grams per 100 cc. of fluid |                       |                |            |                |         |           |       |                              |                                 |
| Normal serum                    | 8.300                      | 0.062                 | 3.071          | 1.059      | 2.012          | 5.09    | 0.397     |       | 1:1.7                        | 37.0                            |
| Inflammatory. Unknown etiology* |                            |                       |                |            |                |         |           |       |                              |                                 |
| 20                              | 4.400                      | 0.046                 | 1.780          | 0.470      | 1.310          | 2.620   | 0.362     | 0.650 | 1:1.5                        | 40                              |
| 20a                             | 3.969                      | 0.080                 | 1.882          | 0.392      | 1.490          | 2.087   | 0.355     | 0.650 | 1:1.1                        | 47.6                            |
| 60                              | 3.831                      | 0.050                 | 1.437          | 0.650      | 0.787          | 2.394   | 0.404     |       | 1:1.6                        | 39.3                            |
| 60a                             | 3.412                      | 0.045                 | 2.537          | 0.569      | 1.968          | 0.875   | 0.412     |       | 1:0.34                       | 74.3                            |
| 54                              | 4.781                      | 0.067                 | 2.056          | 0.881      | 1.175          | 2.715   | 0.397     |       | 1:1.3                        | 43                              |
| 80                              | 5.487                      | 0.049                 | 2.625          | 0.822      | 1.803          | 2.862   | 0.412     |       | 1:1.1                        | 47.6                            |
| 204                             | 6.775                      | 0.022                 | 2.187          | 4.588      |                |         | 0.391     |       | 1:2.14                       | 32                              |
| Inflammatory. Tuberculosis      |                            |                       |                |            |                |         |           |       |                              |                                 |
| 52                              | 4.275                      | 0.022                 | 2.387          | 1.181      | 1.206          | 1.888   | 0.372     |       | 1:0.8                        | 56                              |
| 53                              | 6.250                      | 0.056                 | 2.969          | 0.812      | 2.157          | 3.291   | 0.376     |       | 1:1.1                        | 47.6                            |
| 206                             | 5.231                      | 0.039                 | 3.506          |            |                | 1.725   | 0.405     |       | 1:0.48                       | 67                              |
| Inflammatory. Empyema           |                            |                       |                |            |                |         |           |       |                              |                                 |
| 30                              | 5.019                      | 0.086                 | 1.027          | 0.625      | 0.402          | 3.992   | 0.372     |       | 1:3.8                        | 20.8                            |
| Non-inflammatory. Nephritis     |                            |                       |                |            |                |         |           |       |                              |                                 |
| 56                              | 3.581                      | 0.060                 | 1.469          | 0.631      | 0.838          | 2.112   | 0.426     |       | 1:1.4                        | 41                              |
| 205                             | 1.250                      | 0.164                 | 0.875          |            |                | 0.375   | 0.426     |       | 1:0.3                        | 70                              |
| Non-inflammatory. Neoplasms     |                            |                       |                |            |                |         |           |       |                              |                                 |
| 63                              | 4.250                      | 0.053                 | 1.776          | 0.490      | 1.280          | 2.484   | 0.390     |       | 1:1.4                        | 41.8                            |
| 202                             | 5.023                      | 0.038                 |                |            |                |         | 0.440     |       |                              |                                 |
| 203                             | 4.950                      | 0.048                 |                |            |                |         | 0.433     |       |                              |                                 |

\* It will be noted that the first four sets of figures (20 and 20a, 60 and 60a) are each two tappings at a few days interval, indicating the changes that may take place in the fluid in the same individual.

The three tuberculous exudates, it will be seen, resemble the other inflammatory exudates. Tuberculous exudate no. 53,

which had nearly as much protein as has blood serum, had a large fibrinogen content, agreeing with the statement of Oswald that fibrinogen is the last of the proteins to leave the blood vessels.

*Abdominal effusions*

| CASE NUMBER | TOTAL PROTEIN              | INCOAGULABLE<br>IN TROGEN | TOTAL GLOBULIN | EUGLOBULIN | PSEUDOGLOBULIN | ALBUMIN | CHLORIDES | TOTAL SOLIDS | ASH | RATIO OF GLOBULIN TO<br>ALBUMIN | PER CENT OF GLOBULIN<br>IN PROTEIN |
|-------------|----------------------------|---------------------------|----------------|------------|----------------|---------|-----------|--------------|-----|---------------------------------|------------------------------------|
|             | Grams per 100 cc. of fluid |                           |                |            |                |         |           |              |     |                                 |                                    |

*Tuberculous and new growths*

|     |       |       |       |  |  |       |       |  |  |        |      |
|-----|-------|-------|-------|--|--|-------|-------|--|--|--------|------|
| 105 | 1.725 | 0.045 | 0.281 |  |  | 1.444 | 0.398 |  |  | 1:5.1  | 16.3 |
| 209 | 3.725 | 0.040 | 1.838 |  |  | 1.887 | 0.385 |  |  | 1:1.0+ | 49   |
| 208 | 3.681 | 0.043 | 1.462 |  |  | 2.219 | 0.418 |  |  | 1:1.5  | 39.7 |

*Cardiac*

|     |       |       |       |       |       |       |       |       |       |        |      |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|------|
| 7   | 2.080 | 0.037 | 0.882 | 0.189 | 0.693 | 1.198 | 0.362 | 3.400 | 0.958 | 1:1.36 | 42.3 |
| 28  | 1.567 | 0.049 | 0.692 | 0.275 | 0.417 | 0.975 | 0.454 |       |       | 1:1.4  | 62   |
| 40  | 3.675 | 0.084 | 1.919 | 0.625 | 1.294 | 1.756 | 0.412 |       |       | 1:0.9  | 52.2 |
| 69  | 4.694 | 0.055 | 1.787 | 0.750 | 1.037 | 2.907 | 0.376 |       |       | 1:1.6  | 38   |
| 81  | 4.712 | 0.055 | 1.656 | 0.456 | 1.200 | 3.056 | 0.404 |       |       | 1:1.9  | 34   |
| 32  | 3.230 | 0.053 | 1.062 | 0.372 | 0.690 | 2.168 | 0.412 |       |       | 1:2    | 33.3 |
| 66  | 3.702 | 0.076 | 1.462 | 0.500 | 0.962 | 2.240 | 0.418 |       |       | 1:1.5  | 39.9 |
| 68  | 3.123 | 0.077 | 1.331 | 0.463 | 0.868 | 1.792 | 0.404 |       |       | 1:1.3  | 42.6 |
| 207 | 3.381 | 0.040 |       |       |       | 0.440 |       |       |       |        |      |

*Cirrhosis of the liver*

|    |       |       |       |       |       |       |       |       |       |        |      |
|----|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|------|
| 15 | 3.332 | 0.090 | 1.625 | 0.525 | 1.100 | 1.707 | 0.428 | 4.815 | 0.950 | 1:1.05 | 49   |
| 26 | 3.017 | 0.051 | 1.012 | 0.205 | 0.707 | 2.005 | 0.390 |       |       | 1:2.0  | 33.3 |
| 67 | 0.521 | 0.089 | 0.325 | 0.125 | 0.200 | 0.193 | 0.326 |       |       | 1:0.6  | 62.3 |
| 79 | 0.686 | 0.038 | 0.400 | 0.131 | 0.769 | 0.256 | 0.497 |       |       | 1:0.6  | 62.3 |

*Nephritis*

|    |       |       |       |  |  |       |       |       |  |     |
|----|-------|-------|-------|--|--|-------|-------|-------|--|-----|
| 1a | 0.285 | 0.035 | 0.285 |  |  | 0.420 | 1.040 | 0.875 |  | 100 |
|----|-------|-------|-------|--|--|-------|-------|-------|--|-----|

*Cardionephritis*

|    |       |       |       |       |       |       |       |  |  |       |      |
|----|-------|-------|-------|-------|-------|-------|-------|--|--|-------|------|
| 32 | 3.230 | 0.053 | 1.062 | 0.372 | 0.690 | 2.168 | 0.412 |  |  | 1:2   | 33.3 |
| 66 | 3.702 | 0.076 | 1.462 | 0.500 | 0.962 | 2.240 | 0.418 |  |  | 1:1.5 | 39.9 |
| 68 | 3.123 | 0.077 | 1.331 | 0.463 | 0.868 | 1.792 | 0.404 |  |  | 1:1.3 | 42.6 |

In the tuberculous fluid (105) the protein content is moderate in amount, but very much less in this particular case, than that found in pleural fluids of similar origin. The percentage of globulin is much less than that of blood serum. The chloride content approximates the values obtained in the blood.

### *Absorption of effusions*

Landsberg<sup>20</sup> found that in exudates that were being absorbed the proportion of protein to amino nitrogen falls, indicating that digestion of the proteins precedes and accounts for the absorption. This digestion he attributes to the serosa rather than to the leucocytes of the effusion, since the more leucocytes an effusion contains and the more injury to the serosa, the less the rate of absorption. Natali<sup>21</sup> found no albumose or peptone in absorbing exudates and therefore concluded that absorption occurred without preliminary proteolysis, but apparently amino acids were not sought. This author found that after aspiration of tuberculous effusion the new-formed fluid is less dense and not so rich in chlorides and proteins, especially in globulin. During spontaneous absorption the fluid may become poorer in salts and proteins even while the globulin is increasing, or it may become more dense and richer in all components, especially the globulin. The relative increase in the globulin is to be expected in view of its known resistance to proteolysis, and the fact that the globulin molecule is larger than that of the albumin.

### *Differentiation of exudates and transudates*

Since tuberculous effusions represent fluids of intermediate protein richness, they may appear on the border-line of transudates and exudates, but generally they are frankly exudates, especially pleural and peritoneal fluids. Rivalta<sup>22</sup> has suggested the following test to distinguish exudates and transudates: Into a beaker containing 200 cc. of water with 4 drops of

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<sup>20</sup> Wien. Arch. inn. Med., 1921 (2), 467.

<sup>21</sup> Riv. crit. di clin. med., 1921 (22), 337, 349; abst. in Zent. f. Tuberk., 1922 (17), 169.

<sup>22</sup> Rif. Med., 1903; Biochem. Centr. 1904 (2), 529.

glacial acetic acid, let fall a few drops of the fluid to be tested. If an exudate, a bluish-white line is left transiently behind the sinking drops, due to precipitation of proteins. Ujihard believes that the precipitate is a mixture of euglobulin and fibrinoglobulin, agreeing with Joachim, although earlier authors had considered it to be nucleoprotein. This test, and also certain modifications,<sup>23</sup> seem to give quite reliable results.<sup>24</sup> With tuberculous effusions Rivalta's test is positive, but not Morelli's test, which consists in dropping the fluid into saturated  $HgCl_2$  solution, a yellowish ring of albuminate forming with non-tuberculous exudates, and a granular precipitate with transudates.<sup>25</sup>

Memmi<sup>26</sup> suggests the larger content of lipase as a means of distinction of exudates. Tedeschi<sup>27</sup> states that egg-albumen fed in large amounts appears in transudates and not in exudates, and can be detected by the biological precipitin test. Sugar is found more often in transudates.<sup>28</sup>

Another test, recommended by Müller, is to place 1 or 2 drops of the fluid on the surface of a small quantity of Millon's reagent; with non-tuberculous exudates there forms a granular disk on the surface which disintegrates readily and the fluid is colored red (presumably from tyrosine previously liberated by autolysis) while tuberculous exudates form a firm film without coloration of the fluid. However, tuberculous exudates from patients who have been given iodides may show a positive red coloration because of the heightened autolysis.<sup>29</sup>

### *Non-protein organic contents*

Proteoses,<sup>29</sup> leucine, and tyrosine may be present in small quantities in exudates that contain many leucocytes, being produced by autolysis (Umber); therefore, we should expect them to be abundant in tuberculous empyema, but not in clear serous exudates, but no analytical data are available.

Landsberg,<sup>30</sup> in studying the process of absorption of pleural effusion, gives figures for protein and total amino nitrogen obtained by several analyses of the same effusion in cases of tuberculosis of the pleura, as well as other conditions.

<sup>23</sup> See Rivalta, *Policlinico*, 1910 (17), 676.

<sup>24</sup> See Ujihard, *Berl. klin. Woch.*, 1914 (51), 1112, Bibliography.

<sup>25</sup> See Zannini, *Gaz. degli Osped.*, 1914 (4), 461.

<sup>26</sup> *Clin. Med., Ital.*, 1905, No. 3.

<sup>27</sup> *Gaz. degli Osped.*, 1905 (26), 88.

<sup>28</sup> See Mayer, *Beitr. Klin. Tuberk.*, 1913 (29), 73.

<sup>29</sup> Opie, *Jour. Exp. Med.*, 1907 (9), 391.

| LESION                          | PROTEIN   | AMINO-N       |
|---------------------------------|-----------|---------------|
|                                 |           | per cent      |
| Tuberculous, serofibrinous..... | 2.5 -3.03 | 0.0195-0.0238 |
| Tuberculous, serofibrinous..... | 4.3 -4.5  | 0.0302-0.0320 |
| Tuberculous, purulent.....      | 4.5 -4.6  | 0.033 -0.0346 |
| Tuberculous, serous.....        | 3.1 -4.2  | 0.021 -0.0287 |
| Tuberculous, serous.....        | 3.3 -3.4  | 0.0244-0.0250 |
| Tuberculous, serous.....        | 4.6 -4.84 | 0.0281-0.0324 |
| Tuberculous, purulent.....      | 4.1 -4.23 | 0.0242-0.0251 |
| Pneumonic.....                  | 4.1 -4.6  | 0.0361-0.0448 |
| Pneumonic.....                  | 4.35-5.08 | 0.0276-0.0308 |
| Pneumonic.....                  | 4.0 -4.62 | 0.0308-0.0330 |
| Rheumatic.....                  | 4.5 -4.65 | 0.0285-0.0290 |

It will be seen that the figures in tuberculosis are not essentially different from those in other conditions.

Nucleoproteins may be present from leucocytic disintegration in exudates, as well as the products of their further splitting, such as purines and phosphates. Galdi and Appiani<sup>30</sup> found uric acid constantly in amounts between 0.0055 grams and 0.0714 grams, in all exudates, of which 7 were tuberculous and 2 neoplastic. In 3 transudates amounts from 0.006 to 0.011 grams were found.

All the other innumerable components of plasma may be found in edematous fluids; thus sugar and urea (Carrière)<sup>30</sup> are present, as well as other extractives. The amount of urea varies quite as it does in the blood of the same individual,<sup>31</sup> and it seems probable that all the crystalloid substances present in the blood pass freely into and from inflammatory exudates, so that an equilibrium between blood and exudates is approximated.<sup>32</sup> However, there is some evidence that substances injected into the pleural cavity are absorbed less rapidly with tuberculous exudates than

<sup>30</sup> Riforma Med., 1904, p. 1373; also Carrière, Compt. Rend. Soc. Biol., 1899 (51), 467.

<sup>31</sup> Javal and Adler, Compt. Rend. Soc. Biol., 1906 (61), 235; Rosenberg, Berl. klin. Woch., 1916 (53), 1314.

<sup>32</sup> Wells and Hedenburg, Jour. Infect. Dis., 1912 (11), 349; Scheel, Nord. Med. Laeg., 1916 (77), 610.

with simple effusion,<sup>33</sup> presumably because of the mechanical obstacle of the layer of fibrin and tuberculous granulation tissue on the pleural surfaces.<sup>34</sup>

Sugar is said sometimes to be greater in amount in transudates than in the blood, but in exudates it is usually, if not always, lower than 0.1 per cent.<sup>35</sup>

By using more accurate methods than have been employed by most of the observers quoted above, Denis and Minot<sup>2</sup> found urea, uric acid and creatinine to occur in exudates and transudates in the same concentrations as in the blood, but the sugar content of ascitic fluids is somewhat higher than that of the blood. Creatine, fats and cholesterol are much lower in transudates than in exudates in which they approach the concentration in the blood. In ascitic fluid, the urea, uric acid and cholesterol are influenced by the diet. Their figures for tuberculous fluids are as follows, in milligrams per 100 cc.:

|                              | SPECIFIC GRAVITY | TOTAL PROTEINS | NON-PROTEIN NITROGEN | UREA-N | CREATINE + CREATININE | TOTAL FAT | TOTAL CHOLESTEROL | SUGAR | URIC ACID |
|------------------------------|------------------|----------------|----------------------|--------|-----------------------|-----------|-------------------|-------|-----------|
| Tuberculous peritonitis..... | 1040             | 3,375          | 38                   | 13     | 6.0                   | 372       | 77                | 86    |           |
| Pleurisy.....                | 1032             | 3,981          | 36                   |        | 6.6                   | 330       | 115               | 110   |           |
| Tuberculous peritonitis..... | 1020             | 4,919          | 31                   | 15     | 4.8                   | 408       | 89                | 141   | 2.8       |
| Blood from no. 3.....        |                  |                | 30                   | 12     | 7.7                   | 560       | 208               | 140   |           |
| Tuberculous pleurisy.....    | 1022             | 4,548          | 22                   | 11     | 6.8                   | 650       | 108               | 95    | 2.5       |
| Blood from no. 4.....        |                  |                | 25                   | 12     | 8.9                   | 910       | 250               | 90    | 2.0       |

Max Rosenberg<sup>31</sup> also found that the figures for the blood and tuberculous exudates of the same patient were similar to each other, as follows:

<sup>33</sup> See Mayer, Beitr. Klin. Tuberk., 1913 (29), 74.

<sup>34</sup> Cobet and Ganter, Deut. Arch. klin. Med., 1921 (135), 146.

<sup>35</sup> Hegler and Schumm, Med. Klinik, 1913 (9), 1810.

|                                     | UREA GRAM<br>PER M | CREATININE<br>MG.M. PER M | INDICAN<br>CC. GIVING +<br>REACTION |
|-------------------------------------|--------------------|---------------------------|-------------------------------------|
| Tuberculous peritonitis—blood ....  | 0.18               | 12.2                      | 4.0                                 |
| Tuberculous peritonitis—fluid ..... | 0.16               | 13.5                      | 4.0                                 |
| Tuberculous pleuritis—blood.....    | 0.27               | 11.1                      | 10.0                                |
| Tuberculous pleuritis—fluid .....   | 0.25               | 12.2                      | 10.0                                |
| Tuberculous pleuritis—blood .....   | 0.22               | 9.9                       | 8.0                                 |
| Tuberculous pleuritis—fluid.....    | 0.22               | 10.0                      | 8.0                                 |

In exudates, glycogen is found in the leucocytes as long as they retain their vitality, but disappears soon after retrogressive changes begin; hence it is not usually present in old sterile pus. Loeper<sup>36</sup> made quantitative estimates of the glycogen in exudates, finding from 0.59 to 0.62 gram per liter in cellular pneumococcus pleural effusion, 0.25 gram in cellular tuberculous effusion, but only traces in serous tuberculous effusion and in an old tuberculous pyothorax. A pneumonic lung contained 0.85 gram of glycogen per kilo, and traces were found in pneumonic sputum and in the contents of tuberculous cavities.

### *Lipins*

Lecithin is always present, partly bound to globulin and partly free (Christen).<sup>37</sup> Cholesterol is present particularly in fluids that have been standing for a long time in the body, appearing often as visible crystals shining in the fluid; it probably originates from degenerating cells. Ruppert has described a case of pleural effusion of unknown etiology with 1.129 per cent of cholesterol when tapped the first time, 0.22 per cent the second and 0.05 per cent the third. Hedstrom reported finding in an old pleural effusion, 4.5 per cent of cholesterol; one year later there was but 0.09 per cent. Zunz<sup>38</sup> has described a carefully studied case in which 14 aspirations were made; the cholesterol content was about 3 per cent at first, but fell suddenly to 0.48 per cent and then remained between 0.5 per cent and 0.28 per cent. Lecithin varied from 0.1 to 0.04 per cent. As there did not seem to be enough

<sup>36</sup> Arch. Méd. Exp., 1902 (14), 576.

<sup>37</sup> Cent. inn. Med., 1905 (26), 329.

<sup>38</sup> Travaux Ambulance de L'Ocean, La Panne, 1918, Tome II, Fasc. 1.

cells present in the fluid to have yielded the obtained cholesterol through their disintegration, Zunz suggests that it may have been secreted by the walls of the cavity. Weems<sup>39</sup> has reported a similar case, with 1.39 per cent in the first fluid drawn, but smaller amounts in fluids withdrawn later; this patient had a marked hypercholesterolemia. Presumably these were cases of tuberculous pleurisy, but this is not established. Arnell<sup>40</sup> found 0.41 per cent of cholesterol in a tuberculous pleurisy. A pleural effusion of unknown etiology with 9.73 per cent cholesterol has been described by Schulman.<sup>41</sup> In most of these cases some fats have been present, Weems finding 0.33 per cent and Ruppert 0.36 per cent.

The fluid of vesicles is said to contain less cholesterol in tuberculous patients than in other conditions.<sup>42</sup>

#### *Enzymes and immune bodies*

All the enzymes of the plasma may be present, being in all cases probably more abundant in exudates than in transudates. According to Carrière<sup>43</sup> oxidases are inconstant, even in exudates. Lipase is said to be much more abundant in exudates than in transudates<sup>44</sup> and especially in tuberculous effusions, presumably since it is introduced chiefly in the lymphoid cells.<sup>45</sup> Kollert and Frisch<sup>46</sup> state that tuberculous effusions usually show about half the lipase (tributyrin) activity of the blood of the same patient.

Garnier<sup>47</sup> found that while exudates usually contain monobutyrylase corresponding in amount to that in the blood serum, it is generally low in tuberculous pleurisy. The serum of tuberculous pleural exudates lacks the antiprotease activity of blood

<sup>39</sup> Amer. Jour. Med. Sci., 1918 (156), 20.

<sup>40</sup> Hygiea, 1917 (79), 737.

<sup>41</sup> Jour. Amer. Med. Assoc., 1917 (68), 1256.

<sup>42</sup> Ferré, Mauriac, Defaye, Compt. Rend. Soc. Biol., 1912 (73), 141.

<sup>43</sup> Compt. Rend. Soc. Biol., 1899 (51), 561.

<sup>44</sup> Zeri, II Policlinico, 1903 (10), No. 11; Memmi, Clin. Med. Ital., 1905, No. 3; Galletta, Clin. med. Ital., 1911 (50), 143.

<sup>45</sup> Fiessinger and Marie, Compt. Rend. Soc. Biol., 1909 (67), 177.

<sup>46</sup> Beitr. Klin. Tuberk., 1920 (43), 305.

<sup>47</sup> Compt. Rend. Soc. Biol., 1903 (55), 1557.

serum, and is itself capable of digesting coagulated protein in a weakly acid medium, because of an enzyme derived from the large mononuclear cells (Opie).<sup>48</sup> If large numbers of polynuclear leucocytes are present they yield an enzyme which will digest in neutral or faintly alkaline fluids such as the serous exudates are, and then the usual changes of suppuration take place.

*Peptid-splitting enzymes* are usually found in such fluids,<sup>49</sup> especially tuberculous exudates,<sup>50</sup> and these enzymes seem to be different from both erepsin and trypsin. Mandelbaum<sup>51</sup> states that while not all tuberculous exudates show a high capacity to split glycyl-tryptophane, generally they give positive reactions at 1-20 dilution, while other pleural and peritoneal exudates and transudates are usually negative at dilutions of 1-2. Probably this type of enzyme is more often present than trypsin.

The various *immune bodies*, cytotoxins, hemolysins, agglutinins, etc., seem to pass freely into both transudates and exudates, and their presence is not characteristic of either,<sup>52</sup> but as a rule the proportion is much higher in exudates.<sup>53</sup> Purulent fluids are usually poor in opsonins,<sup>54</sup> in nonpurulent fluids the opsonin content varies with the amount of proteins.<sup>55</sup> Exudates usually contain about as much complement as the serum, but in suppuration the complement disappears; transudates contain little of either complement or hemolysins.<sup>56</sup>

C. Delrez<sup>57</sup> reports that only the acute or subacute serous exudates are rich in alexin, while sero-purulent exudates contain much less, and the purulent exudates are almost entirely lacking in alexin, but no sharp distinction can be made between transudates and exudates on the basis of alexin content alone.

<sup>48</sup> Jour. Exp. Med., 1905 (7), 316.

<sup>49</sup> Hall and Williamson, Jour. Path. and Bact., 1911 (15), 351.

<sup>50</sup> See H. Koch, Zeit. Kinderheilk., 1914 (10), 1.

<sup>51</sup> Münch. med. Woch., 1914 (61), 461.

<sup>52</sup> Granström, Inaug. Dissert., St. Petersburg, 1905.

<sup>53</sup> Not corroborated by Lüdke, Cent. f. Bakt., 1907 (44), 268.

<sup>54</sup> Opie, Jour. Exper. Med., 1907 (9), 515.

<sup>55</sup> Böhme, Deut. Arch. klin. Med., 1909 (96), 195.

<sup>56</sup> Aronstamm, Cent. f. Bakt., 1914 (74), 326.

<sup>57</sup> Bull. Acad. royal méd. Belgique, 1919 (29), 733.

*Precipitin reactions, etc.*

Tuberculous effusions have been often used as a source of material in immunizing animals against human proteins. The precipitins thus formed are specific for human serum or for the proteins of the effusion, but cannot be used to differentiate a tuberculous from a non-tuberculous exudate, or a pleural fluid from an ascitic fluid (Quadrone).<sup>58</sup>

CHYLOUS EFFUSIONS<sup>59</sup>

Fat may be present in effusions in sufficient quantity to cause a milky appearance, either from escape of chyle from a ruptured or obstructed thoracic duct, or through fatty degeneration of the cells in the effusion or the lining of the walls of the cavity. The former are designated as chylous, the others as *chyliform* or *adipose* fluids, but it is not always easy to distinguish between them. Either form may be the result of tuberculous processes, especially the chyliform,<sup>60</sup> 17 of Boston's<sup>61</sup> 128 cases being attributed to tuberculosis. The composition of the fluids in true chylous exudates will vary according to the food taken and the amount of fat the food contains, and will resemble the composition of chyle, except to the extent that it is modified by the effusion or absorption going on in the cavity. They are characterized by strong bactericidal powers as evidenced by lack of putrefaction after long standing.

Analyses of human chyle are scanty. Panzer<sup>62</sup> found 90.29 to 94.53 per cent water; 5.47 to 9.71 per cent solids; 0.80 to 1.04 per cent inorganic salts; 2.16 per cent coagulable protein; 6.59 per cent ether-soluble material; also diastatic enzyme, soaps, and occasionally traces of cholesterol, lecithin, and sugar. Carlier,<sup>63</sup> in a specimen from a child, obtained very similar results, except that the salts were much less abundant. The proteins

<sup>58</sup> Cent. f. Bakt. (Ref.), 1905 (36), 270.

<sup>59</sup> Literature by Gandin, Ergeb. inn. Med., 1913 (12), 218.

<sup>60</sup> See Rotmann, Zeit. klin. Med., 1897 (31), 416.

<sup>61</sup> Jour. Amer. Med. Assoc., 1905 (44), 513.

<sup>62</sup> Zeit. physiol. Chem., 1900 (30), 113.

<sup>63</sup> British Med. Jour., 1902 (ii) 175.

and fats vary greatly with the diet; thus Sollmann<sup>64</sup> found variations in the proteins from 1.85 to 6.5 per cent.

Chylous ascites fluid often, but not always, contains sugar,<sup>65</sup> but it may disappear after having once been present; the amount of fat is small, usually about 1 per cent, and the fluid is rich in solids. If due to a ruptured thoracic duct, it may be possible to detect special fats taken in the food, e.g., butter-fats (Straus).<sup>66</sup> The reaction is usually alkaline or neutral, and some specimens coagulate spontaneously. Specific gravity varies from 1.007 to 1.040, the average being about 1.017. Perhaps the most important characteristic is the variation produced by changes in diet.<sup>67</sup> Zdarek<sup>68</sup> found in a chyle-cyst 2.7 per cent of fats, 7.2 per cent of proteins, and 0.05 per cent of sugar; feeding of fats increased their amount in the cyst and starvation decreased it. Schumm<sup>69</sup> found in the solids of such a cyst 35.76 per cent of fat, some of which was in the form of calcium soap.

*Chylothorax* fluid is, of course, quite similar to that of chylous ascites. Thus, Buchtala<sup>70</sup> found 91.34 per cent of water, 8.66 per cent solid, 4.86 per cent protein, 2.5 per cent fat, 0.26 per cent cholesterol, and 0.94 per cent ash. Similar figures were obtained by Salkowski<sup>71</sup> and others.

The variations which the fluid withdrawn from the same patient may show is illustrated by the analyses by Patein<sup>71</sup> of three similar liquids removed from the chest of a tuberculous patient

<sup>64</sup> Amer. Jour. Physiol., 1907 (17), 487; see also Hamill, Jour. Physiol., 1906 (35), 151.

<sup>65</sup> For example, v. Tabora (Deut. med. Woch., 1904 (30), 1595) found as high as 0.864 per cent of sugar in a typical case.

<sup>66</sup> Arch. Physiol. et Pathol., 1886 (Ser. 3, vol. 8), 367.

<sup>67</sup> A sample of the composition of 1 liter of chylous ascitic fluid is shown by the analysis in the case studied by Comey and McKibben (Boston Med. and Surg. Jour., 1903 (148), 109): Specific gravity, 1.010; solids, 21 gm.; protein, 9.75 gm.; urea, 1.28 gm.; fat, 1.45 gm.; inorganic matter, 8 gm.; peptone (?) and sugar, present; fibrinogen, mucin, nucleo-albumin, and uric acid absent.

<sup>68</sup> Zeit. f. Heilk., 1906 (27), 1.

<sup>69</sup> Zeit. physiol. Chem., 1906 (49), 266.

<sup>70</sup> Zeit. physiol. Chem., 1910 (67), 42.

<sup>71</sup> Virchow's Arch., 1909 (198), 189; also Tuley and Graves, Jour. Amer. Med. Assoc., 1916 (66), 1844; Patein, Jour. Pharm. Chim., 1915 (11), 265.

at different periods. The first, amounting to 1 liter, was milky, slightly yellowish, free from pus, of alkaline reaction, specific gravity 1.025; in grams per liter, total solids 85.8, mineral matter 9.5, fatty matter 2.0. The second, 500 cc., was greenish gray, thick, milky, very alkaline, specific gravity 1.021; in grams per liter, total solids 64.5, mineral matter 9.6, fatty matter 8.4, and showed pus and fatty granules. In both liquids were traces of reducing and biliary matters, no fibrin, no fibrinogen. The third liquid was also purulent, greenish yellow, alkaline, specific gravity 1.022, total solids 69.5, mineral matters 8.2, albuminoids 42.5, fatty matter 14.8, consisting of free fatty acids 11.2, fatty acids combined as soap 3.6 grams per liter. Reducing and biliary matters, fibrin and fibrinogen were absent. The fatty matter also contained a small amount of cholesterol and notable amounts of lecithin. The melting point of the fatty substance is much higher than that of the fatty matters from ascitic liquids or chyliform urine, and may exceed 80°. The albuminous matter in the third liquid was nearly all precipitated by adding 10 volumes of water and rendering faintly acid with acetic acid. The precipitate contained no mucin, but nucleoproteins abundantly.

*Ascites adiposus* is characterized by the absence of sugar and by a higher percentage of fat, the maximum observed being 6.4 per cent. It is ascribed to fatty metamorphosis of cells, particularly in carcinomatous and tuberculous exudates. Edwards was able to show experimentally that a transudate may change from serous to cellular, and later come to contain fat.

*Pseudochylous* effusions are also observed, not only in the abdominal and thoracic cavities, but even in the fluid of the edematous legs and scrotum; these resemble chylous fluids in being turbid or milky, but are said to contain little or no fat. The turbidity is ascribed chiefly to lecithin, which is largely combined with the pseudoglobulin of the fluid (Joachim).<sup>72</sup> Possibly in some cases the turbidity is partly or largely (Poljakoff)<sup>73</sup> due to poorly dissolved proteins.

<sup>72</sup> Münch. med. Woch., 1903 (50), 1915; also Christen,<sup>37</sup> Wallis and Schöllerberg, Quart. Jour. Med., 1910 (3), 301; 1911 (4), 153.

<sup>73</sup> Fortschr. d. Med., 1903 (21), 1081; also Haushalter, Compt. Rend. Soc. Biol., 1910 (68), 550.

Hammarsten has observed turbidity due to mucoid substances, as also have Gouraud and Corset.<sup>74</sup> The pseudochylous effusions have a lower freezing point, a lower specific gravity, lower fat and greater lecithin content than typical chylous ascites. Gandin, however, questions the possibility of always differentiating the three types of turbid fluids as above indicated. Collecting all the recorded analyses in the literature he finds wide discrepancies, as indicated in the following table: (The maximum and minimum percentage figures are given for each component determined quantitatively, with the average in parentheses.)

|                  | CHYLOUS          | ADIPOSE (CHYLIFORM) | PSEUDOCHYLOUS      |
|------------------|------------------|---------------------|--------------------|
| Ether extract .. | 0.065-9.2 (1.65) | 0.1-4.3 (1.15)      | 0.007-1.86 (0.25)  |
| Cholesterol ...  | + in 7, - in 2   | + in 4              | + in 3, - in 2     |
| Lecithin.....    | + in 4, - in 1   | + in 3              | + in 20, - in 2    |
| Sugar.....       | + in 46, - in 28 | + in 1, - in 4      | + in 15, - in 14   |
| Dry residue..... | 3.1 - 10.6 (6.2) | 1.6 - 11.7 (5.1)    | 1.2 - 7.6 (2.9)    |
| Protein.....     | 0.9 - 7.7 (3.5)  | 0.6 - 6.8 (3.0)     | 0.1 - 4.2 (1.4)    |
| "Pepton".....    | + in 6, - in 4   | + in 1, - in 2      | + in 1, - in 5     |
| Ash.....         | 0.1 - 1.0 (0.59) | 0.45 - 1.03 (0.65)  | 0.49 - 0.90 (0.73) |

It is quite evident that although the pseudochylous fluids usually contain little fat, they often contain more than the minimal content found in the other forms. Each type of fluid overlaps the others in one respect or another. Gandin states that to produce a turbid fluid but 0.01 to 0.1 per cent of finely emulsionized fat is necessary, and he believes that milky fluids always mean admixture of chyle, rejecting the terms pseudochylous and chyliform as unwarranted. He admits that fluids may contain droplets of fats not emulsionized, and hence not milky, which may be properly called adipose fluids. There are no characteristic chemical differences in the fats extracted from the different types of fluids.

#### CHEMISTRY OF PNEUMOTHORAX

By far the commonest cause of non-traumatic pneumothorax is pulmonary tuberculosis, in 715 of 918 cases collected by Biach.<sup>75</sup>

<sup>74</sup> Compt. Rend. Soc. Biol., 1906 (60), 23.

<sup>75</sup> Wien. med. Woch., 1880 (30), 6 and 38.

The practice of artificial pneumothorax therapy has added much to the interest in this subject.

The composition of the gases found in the pleural cavity in pneumothorax will necessarily vary greatly according to the cause. If the pleural cavity is in free communication with the exterior, the gas will be simply slightly modified air; for example, Ewald<sup>76</sup> found the following proportions in the gases in such a pneumothorax: CO<sub>2</sub>, 1.76 per cent; O, 18.93 per cent; and 79.31 per cent N. Here the proportion of CO<sub>2</sub> is even a little less than in ordinary expired air which contains 3.3 to 3.5 per cent. In a seropneumothorax Ewald found 8.13 per cent of CO<sub>2</sub>, 1.26 per cent of O, and 90.61 per cent of N, which is quite similar to the proportions of the gases in dry pneumothorax. When air enters a closed pleural cavity and no effusion follows, it is slowly absorbed until a mixture of about 90 per cent N, 4 per cent O and 6 per cent CO<sub>2</sub> results; but if there is a serous effusion the oxygen disappears nearly or quite completely (Tobiesen),<sup>77</sup> as shown in the following table of illustrative cases:

*Change in atmospheric air injected into pleural cavity*

| TOTAL QUANTITY OF INJECTED AIR<br><i>ccm.</i> | DAYS AFTER LAST INJECTION | SIZE OF LAST INJECTION<br><i>ccm.</i> | O <sub>2</sub><br><i>per cent</i> | CO <sub>2</sub><br><i>per cent</i> | N <sub>2</sub><br><i>per cent</i> | REMARKS             |
|---|---------------------------|---------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|---------------------|
| 4,600   | 9                         | 500                                   | 3.32                              | 6.29                               | 91.39                             | Pleura normal       |
| 4,100   | 12                        | 400                                   | 0.09                              | 9.28                               | 90.63                             | Exudative pleuritis |
| 4,900   | 30                        | 300                                   | 0.10                              | 10.03                              | 89.87                             | Exudative pleuritis |
| 1,300   | 8                         | 400                                   | 3.47                              | 6.26                               | 90.27                             | Pleura normal       |
| 3,400   | 13                        | 600                                   | 0.12                              | 9.37                               | 90.66                             | Exudative pleuritis |

Furthermore, it makes no difference whether O, N or CO<sub>2</sub> is injected into the pleural cavity, after a day or two one finds approximately the same mixture, approaching a proportion of 90 per cent N, 6.7 per cent CO<sub>2</sub>, and 3.4 per cent O as long as there is no pleuritis; with pleuritis the oxygen tends to disappear,

<sup>76</sup> Complete literature and résumé given by Clemens, in Ott's "Chem. Path. der Tuberkulose," Berlin, 1903, p. 406.

<sup>77</sup> Beitr., Klin. Tuberk., 1911 (19), 451; 1911 (21), 109; Deut. Arch. klin. Med., 1914 (115), 399.

and as this change is recognizable earlier than the exudate it may be of some diagnostic value (Tobiesen). Tachau and Thilenius<sup>78</sup> corroborated the above, finding when there was no exudate the CO<sub>2</sub> content to be from 6.5 to 8.5, with O from 1.9 to 6.2; with an exudate the figures were CO<sub>2</sub>, 10.5 to 12.7; oxygen, less than 1 per cent. Grass<sup>79</sup> found similar amounts, but says that if the exudate is small or the result of operative trauma, the figures may be the same as without exudate. When air is injected the CO<sub>2</sub> content immediately after is 1.88, after five minutes 2.80, after ten minutes 3.63 per cent showing how rapid is the escape of CO<sub>2</sub> from the tissues into the fluid.

Purulent pneumothorax generally shows more CO<sub>2</sub> than the serous form, the average in the former being 15 to 20 per cent, in the latter 7.5 to 11.5 per cent. The average of the analyses in 6 cases of pyopneumothorax is given by Ewald as 18.13 per cent CO<sub>2</sub>, 2.6 per cent O, and 79.81 per cent N. In open pyopneumothorax the gas approaches more closely the composition of air, but usually shows a slight excess of CO<sub>2</sub>; it is thus possible by a determination of the carbon dioxide to ascertain quite accurately whether a given pneumothorax is in communication with the outside air. The transformation of a purulent into a putrid pneumothorax is accompanied by an increase of CO<sub>2</sub>, even as high as 40 per cent having been found. The products of decomposition by the putrefactive saprophytes also are present, one analysis having shown 4.3 per cent of hydrogen, 6.25 per cent of methane, and traces of hydrogen sulphide.

Infection of a pleural effusion by gas-producing organisms may also convert it into a pneumothorax, although this is not a common occurrence. The gases then present are the same as the organisms produce in similar culture-media, modified somewhat by absorption. The anaërobic gas-producing organisms have been found as the cause of such gaseous accumulations; it is questionable if the ordinary pathogenic organisms can cause a pneumothorax, since they are for the most part not capable of producing gas. The colon bacillus produces gas in sugar-containing media, but the amount of sugar in the pathological exudates is too small to yield any considerable amount of gas; an exception

<sup>78</sup> Zeit. klin. Med., 1914-15 (81-82), 199, 209, 222.

<sup>79</sup> Beitr. Klin. Tuberk., 1920 (46), 46; 1922 (51), 134.

is the pleural effusion in diabetes, and pneumothorax from infection of the pleural effusion in a diabetic by *B. coli* has been reported. Complete quantitative analyses of the gas in this form of pneumothorax seem not to have been made, but May found about 20 per cent of CO<sub>2</sub>. The combustibility of the gas has frequently been noted, and is probably due to hydrogen and methane.

A study by Mayer<sup>80</sup> of the effusion which forms in a large per cent of cases after artificial pneumothorax in pulmonary tuberculosis showed that the protein content increases with time, and also with increased pressure. In general the effusions give positive acetic acid reactions (Moritz-Rivalta test), have higher protein content than simple transudates, and give a negative Millon's reaction, indicating that they are of tuberculous etiology.

#### MENINGEAL EFFUSIONS<sup>81</sup>

Normal meningeal fluid differs from all other serous fluids in being clear and watery, in its low specific gravity (1.006 to 1.007), in containing but a trace of protein, which is chiefly globulin (with a trace of proteose (?)), and 0.05 to 0.13 per cent of a reducing substance that is probably glucose.<sup>82</sup> The total volume of meningeal fluid is normally in the adult from 60 to 150 cc., and Frazier estimates that from 360 to 720 cc. is secreted daily. Halliburton gives the following analyses of pathological accumulations of supposedly normal fluids.

#### *Spina bifida*

|               | CASE 1 | CASE 2  | CASE 3  |
|---------------|--------|---------|---------|
| Water.....    | 989.75 | 989.877 | 991.658 |
| Solids.....   | 10.25  | 10.123  | 8.342   |
| Proteins..... | 0.842  | 1.602   | 0.199   |
| Salts }       | 9.626  | { 0.631 | 3.028   |
| Extractives}  |        | 7.890   | 5.115   |

<sup>80</sup> Beitr. Klin. Tuberk., 1913 (29), 51.

<sup>81</sup> Résumé by Wm. Boyd, "Physiology and Pathology of the Cerebro-spinal Fluid," MacMillan, 1920; Levinson, "Cerebrospinal Fluid," Mosby, 1919; Blatters and Lederer, Jour. Amer. Med. Assoc., 1913 (60), 811; Herrick and Dannenberg, ibid., 1919 (73), 1321; Levinson, Amer. Jour. Dis. Chil., 1919, (18), 568; Becht and Matill, Amer. Jour. Physiol., 1920 (51), 1.

<sup>82</sup> Schloss and Schroeder, Amer. Jour. Dis. Child., 1916 (11), 1; Hopkins, Amer. Jour. Med. Sci., 1915 (150), 847.

### *Reaction*

The alkali reserve is practically the same as that of the blood (McClendon).<sup>83</sup> Kopetsky found that normal spinal fluid is neutral to litmus, and in tuberculous and epidemic meningitis it was acid to phenol phthalein, but by gas chain measurements Levinson<sup>84</sup> found the normal spinal fluid almost neutral ( $\text{pH} = 7.4$  to 7.6); it is quite the same in tuberculous meningitis, but in epidemic meningitis it is 7.3 to 7.4.

### *Physical properties*

According to Fuchs and Rosenthal<sup>85</sup> the average freezing-point of the cerebrospinal fluid is lowered about the same in all diseases ( $\Delta = -0.52^\circ$  to  $-0.54^\circ$ ) except in tuberculous meningitis, where it is much less (average  $-0.43^\circ$ ), but Cohn<sup>10</sup> found the  $\Delta$  to vary from  $-0.474^\circ$  to  $-0.593^\circ$  in 7 cases. The specific gravity varies with the protein content—in tuberculous meningitis it is usually from 1.006 to 1.009. The surface tension is higher than that of the serum and is not characteristically altered in disease according to Kisch and Remertz,<sup>86</sup> but they do not specifically consider tuberculosis. Levinson<sup>81</sup> gives the following table of physicochemical measurements in meningitis.

*Physicochemical findings in various forms of meningitis*

|                        | SPECIFIC GRAVITY   | VIS-COSITY       | FREEZING POINT                             | CONDUC-TIVITY | H-ION CONCEN-TRATION | ALKALINE RESERVE                                      |
|------------------------|--------------------|------------------|--|---------------|----------------------|---|
| Epidemic meningitis    | 1.0075             | 1.0434<br>1.0735 | $-0.55^\circ$ to<br>$-0.57^\circ\text{C}.$ | 0.011629      | pH 7.3               | 18 to 38 per cent of $\text{CO}_2$ bound by the fluid |
| Tuberculous meningitis | 1.00693<br>1.00626 | 1.0693           | $-0.47^\circ$                              | 0.013660      | 7.4-7.6              | 33 to 58 per cent of $\text{CO}_2$                    |

Pathological fluids show also specific alterations in their colloidal property of preventing precipitation of colloidal suspen-

<sup>83</sup> Jour. Amer. Med. Assoc., 1918 (70), 977.

<sup>84</sup> Jour. Infect. Dis., 1917 (21), 556.

<sup>85</sup> Wien. med. Presse., 1904 (45), 2081 and 2135.

<sup>86</sup> Münch. med. Woch., 1914 (20), 1097.

sions by electrolytes (the "Goldzahl" of Zsigmondy),<sup>87</sup> since this depends on the proportion of albumin and globulin in solution. In early stages of tuberculous meningitis the globulin content of the spinal fluid is sometimes altered in such a way that the "gold curve" comes in the so-called syphilitic zone (Eicke, Vogel); later the changes are mostly in the higher tubes. Spät<sup>87</sup> was unable to confirm the statement of Eicke, that there is a characteristic difference in the curves of tuberculous and acute meningitis. Levinson,<sup>81</sup> however, says that in tuberculosis the discoloration is usually greatest in the fifth, sixth and seventh tubes but sometimes it is 7, 8, and 9; in suppurative meningitis the change is in the eighth and ninth tubes. Miller and Levy<sup>88</sup> also found the change most marked in the fifth to seventh tubes.<sup>89</sup>

Among the substitutes suggested for the Lange test is a precipitation of colloidal benzoin. This reaction was not found always to agree with the Lange test in respect to syphilitic spinal fluids by Warnock,<sup>90</sup> and tuberculous meningitis fluids do not precipitate the benzoin in any definite range of dilutions.

#### *Inorganic constituents*

The amount of potassium is about the same as in the blood,<sup>91</sup> and not increased in tuberculous meningitis.<sup>92</sup> Calcium is almost constant at 5 mgm. per 100 cc. or about one-half as much as in the plasma<sup>93</sup> in various diseases, but we have no figures in tuberculosis.

Levinson<sup>81</sup> found that in tuberculous meningitis the chlorides are low, down to 0.5 gram per 100 cc., whereas in suppurative meningitis the figures were near normal, from 0.6 to 0.74 gram.

The changes in P<sub>2</sub>O<sub>5</sub> content in disease are doubtful;<sup>94</sup> in one case of tuberculous meningitis Apelt and Schum<sup>90</sup> found from

<sup>87</sup> Lange, Zeit. Chemother., 1912 (1), 44; Spät, Zeit. Immunität., 1915 (23), 426; Vogel, Arch. Int. Med., 1918 (22), 496.

<sup>88</sup> Bull. Johns Hopkins Hosp., 1914 (25), 133.

<sup>89</sup> Guillain, Laroche and Lechelle (Compt. Rend. Soc. Biol., 1921 (84), 81) have devised a test based on precipitation with colloidal benzoin in a series of tubes with varying concentration, and find specific zones of precipitation in both tuberculosis and syphilis.

<sup>90</sup> Jour. Lab. Clin. Med., 1922 (7), 400.

<sup>91</sup> Myers, Jour. Biol. Chem., 1909 (6), 115, literature.

<sup>92</sup> Rosenbloom and Andrews, Arch. Int. Med., 1914 (14), 536.

<sup>93</sup> Halverson and Bergeim, Jour. Biol. Chem., 1917 (29), 337.

<sup>94</sup> Apelt and Schumm, Arch. Psychiat. u. Nervenkr., 1908 (44), 845.

0.0034 to 0.0049 per cent of  $P_2O_5$ , which is about the same as in most forms of meningitis; twenty-four hours after death the amount had risen to 0.0363 per cent through autolysis. Connall found no difference in the amount of phosphates in tuberculous and epidemic meningitis.

### *Proteins*

Under pathological conditions the amount of protein varies greatly and to some extent characteristically. Thus in tuberculosis, even more constantly than in syphilis, the euglobulin is so greatly increased that it is readily identified by various precipitation methods, as, for example, the butyric acid test of Noguchi<sup>95</sup> and as a rule more or less fibrinogen appears,<sup>96</sup> but sometimes less than in acute luetic meningitis (Kafka).<sup>96</sup>

Mestrezat found the protein content in tuberculous meningitis to vary from 0.12 to 0.56 per cent, and in epidemic meningitis from 0.15 to 0.85 per cent, so that evidently there is no reliable differentiation to be made on the basis of protein content alone. A similar variability was found by Denis and Ayer,<sup>97</sup> who give the following summary from several hundred fluids:

*Protein level in normal and pathologic cerebrospinal fluids*

|  | MG.M. PER 100 CC. |
|--|-------------------|
| Normal.....  | 35-100            |
| Ventricular fluids (brain tumor cases).....                                      | Under 100         |
| Syphilis of the nervous system, inactive.....                                    | 50-125            |
| Active tabes and moderately active syphilis of the nervous system.....           | 100-200           |
| Acute syphilis of the nervous system and general paresis.....                    | 200-600           |
| Lethargic encephalitis.....  | 100-200           |
| Recent cerebral vascular disturbances (hemiplegias, cerebral embolus, etc.)..... | 100-300           |
| Tuberculous meningitis.....  | 200-1000          |
| Acute meningitis.....  | 400-1300          |
| Fluid below spinal cord compression:   |                   |
| "Nonne syndrome".....  | 300-1700          |
| "Fröin syndrome" (1 case).....   | 2010              |

<sup>95</sup> Jour. Exp. Med., 1909 (11), 604.

<sup>96</sup> See Mestrezat, Rev. d. Méd., 1910, p. 189; Kafka, Deut. med. Woch., 1913 (39), 1874.

<sup>97</sup> Arch. Int. Med., 1920 (26), 436.

The figures obtained by Ayer and Foster,<sup>98</sup> given below, are of similar purport.

*Total protein in cerebrospinal fluid*

|  | NUM-<br>BER OF<br>CASES | HIGH                   | LOW                    | AVER-<br>AGE           |
|--|-------------------------|------------------------|------------------------|------------------------|
|  |                         | mgm.<br>per<br>100 cc. | mgm.<br>per<br>100 cc. | mgm.<br>per<br>100 cc. |
| I. Normal (no affection of central nervous system demonstrable)..... | 48                      | 38                     | 16                     | 25                     |
| II. Meningitis:  |                         |                        |                        |                        |
| Tuberculous .....  | 10                      | 252                    | 68                     | 150                    |
| Acute forms (streptococcic and pneumo-<br>coccic).....               | 5                       | 923                    | 64*                    | 364                    |
| "Serous" .....   | 2                       | 87                     | 67                     | 77                     |
| III. Cerebrospinal syphilis:   |                         |                        |                        |                        |
| 1. Meningitic type (average, 253 cells)..                            | 4                       | 118                    | 89                     | 99                     |
| 2. Active (average, 26 cells).....                                   | 12                      | 218                    | 28                     | 73                     |
| 3. Inactive (average, 3.5 cells).....                                | 6                       | 56                     | 26                     | 43                     |

\* Only one case so low; next lowest, 125.

In epidemic meningitis there is more positively charged protein while in tuberculous meningitis there is more negatively charged protein, which can be distinguished by suitable precipitants (Tashiro and Levinson).<sup>99</sup> Thus, in tuberculous meningitis the amount of precipitate with mercuric chloride is usually about twice that with sulphosalicylic acid, whereas in epidemic meningitis the ratio is reversed. Also, a considerable amount of the protein precipitated by sulphosalicylic acid moves toward the cathode in epidemic meningitis, and in tuberculous meningitis it moves toward the anode. Albumose and peptone are absent because of the absence of polynuclear leucocytes.

*Non-protein organic constituents*

According to Rosenbloom<sup>100</sup> there is no creatine or creatinine in the spinal fluid, but Rosenberg<sup>31</sup> found much the same amount of both creatinine and urea as in the blood. There is normally

<sup>98</sup> Jour. Amer. Med. Assoc., 1921 (77), 365.

<sup>99</sup> Jour. Infect. Dis., 1917 (21), 571.

<sup>100</sup> Biochem. Bul., 1916 (5), 22.

from 2 to 4 mgm. of amino-N per 100 cc., or about half that in the blood, without definite changes in disease.<sup>101</sup> The amount of urea is much the same as in the serum of the same person, i.e., 20 to 42 mgm. per 100 cc.<sup>102</sup> and hence about these figures will usually be obtained in tuberculous meningitis. Substances giving the ninhydrin test appear in tuberculous meningitis,<sup>103</sup> and also in other forms of meningitis with considerable protein.<sup>104</sup> Rosenberg states that even with the highest indicanemia no indican is found in the spinal fluid, either normal or meningitic. Of 23 children with tuberculous meningitis, all of whom exhibited more or less acetonuria, all but 2 showed acetone in the cerebro-spinal fluid (Genoese<sup>105</sup>).

The presence of demonstrable tyrosine and tryptophan is said to be characteristic of tuberculous meningitis by Aiello.<sup>105a</sup>

The increased organic matter of tuberculous fluids raises the permanganate reduction index.<sup>106</sup> With a normal index of 2, or at most 2.3, in tuberculous meningitis the figures usually range from 2.5 to 5 and rarely are below 2.5.

Sugar is present in spinal fluid in amounts of from 0.07 to 0.085 per cent and the quantity is not modified significantly in mental diseases;<sup>107</sup> it is reduced in meningitis but increased in uremia.<sup>108</sup> In 122 specimens from 69 cases of tuberculous meningitis, Connall<sup>109</sup> found sufficient sugar present to reduce Fehling's solution in the great majority of the cases at all stages of the disease, but gives no quantitative determinations, and Jacob<sup>110</sup> also found a reducing substance present in tuberculous but absent in acute

<sup>101</sup> Ellis, *et al.*, *Jour. Amer. Med. Assoc.*, 1915 (64), 126.

<sup>102</sup> Ellis and Cullen, *Jour. Biol. Chem.*, 1915 (20), 511.

<sup>103</sup> Nobel, *Münch. med. Woch.*, 1915 (62), 1355, 1786.

<sup>104</sup> Kafka, *Münch. med. Woch.*, 1915 (62), 1355.

<sup>105</sup> *Pediatria, Naples*, 1920 (28), 449.

<sup>105a</sup> *Policlinico*, 1922 (29), 537.

<sup>106</sup> See Hoffman and Schwartz, *Arch. Int. Med.*, 1916 (17), 293.

<sup>107</sup> Weston, *Jour. Med. Res.*, 1916 (35), 199; Kraus and Corneille, *Jour. Lab. Clin. Med.*, 1916 (1), 685.

<sup>108</sup> Leopold and Bernhard, *Amer. Jour. Dis. Child.*, 1917 (13), 34. Discussion of chemistry of spinal fluid in children.

<sup>109</sup> *Quart. Jour. Med.*, 1910 (3), 153.

<sup>110</sup> *Brit. Med. Jour.*, Oct. 26, 1912.

pyogenic meningitis, although in late or healing cases of epidemic meningitis it may be present in sufficient quantities to reduce Fehling's solution. Hopkins<sup>82</sup> gives the figures obtained in 16 cases of tuberculous meningitis, ranging from 0.02 to 0.066 per cent of reducing substance, usually under 0.04 per cent, his normal figures being 0.06 to 0.075 per cent. The blood sugar is usually increased in meningitis, whereas it is decreased in the spinal fluid; thus two cases showed respectively 0.092 and 0.139 per cent in the blood and but 0.029 and 0.027 in the spinal fluid. In diabetes the sugar content is nearly as high in the spinal fluid as in the blood. In acute meningitis Hopkins found generally lower figures than in tuberculosis, but not always. Schloss and Schroeder<sup>82</sup> found in children free from meningeal disease, from 0.05 to 0.134 per cent, approximately the same as for the blood, and in a large proportion of the cases of tuberculous meningitis they found a decrease in the sugar at some stage of the disease, but in a few cases the amount is normal at all times or diminished but slightly. Levinson also obtained low figures in 6 cases of tuberculous meningitis, from 0 to 0.08 per cent, while in 7 cases of suppurative meningitis sugar was demonstrable in only 2 (0.024 and 0.028 per cent).

*Cholesterol* can be found in all cases of mental disease, the amount not bearing any relation to the type of psychosis (Weston);<sup>111</sup> ordinarily 0.2 to 0.7 mgm. per 100 cc. is found. Chauffard,<sup>112</sup> who gives as normal the figure 0.7 to 1.4 mgm., found 1.5 and 2.4 mgm. in 2 cases of tuberculous meningitis.

Levinson,<sup>113</sup> however, states that normal fluid contains no cholesterol, or at most traces. In meningitis, traces are usually found, and in one case of tuberculous meningitis the amount was sufficient for quantitative estimation, 10.2 mgm. per 100 cc.

#### *Enzymes and antibodies*

Peptid-splitting enzymes are especially abundant in tuberculous meningitis.<sup>114</sup> Lenk and Pollak<sup>115</sup> found that normal fluids

<sup>111</sup> Jour. Med. Res., 1915 (33), 119.

<sup>112</sup> Compt. Rend. Soc. Biol., 1911 (70), 855.

<sup>113</sup> Levinson, Landenberger and Howell, Amer. Jour. Med. Sci., 1921 (161), 561.

<sup>114</sup> Major and Nobel, Arch. Int. Med., 1914 (14), 383.

<sup>115</sup> Deut. Arch. klin. Med., 1913 (109), 150.

occasionally split glycyltryptophane but in tuberculous meningitis splitting could usually be obtained in dilutions of 1-100 or 1-200, whereas in epidemic meningitis the fluid was not over one-tenth as active. Mandelbaum<sup>116</sup> confirmed this with cadaver fluid, ascribing the peptidase to the abundant large mononuclear cells. Major and Nobel<sup>114</sup> found occasional cases of tuberculous meningitis in which the activity was not very high (1:1, 1:5, 1:10, etc.) although there were no positive reactions whatever in non-meningitis fluids. H. Koch<sup>50</sup> also found that positive reactions occur only in tuberculous meningitis, but in 6 such cases only 1 gave a reaction with the fluid diluted as much as 1-10.

Levinson and Becht<sup>117</sup> state that the catalytic power of the spinal fluid is determined by the number of cells or the amount of coagulum present, for normal fluid, like cell-free blood serum, contains no catalase. Therefore tuberculous meningitis fluid shows usually less catalase activity than the fluid from suppurative meningitis, but the test has no practical value since the cell count gives more valuable information.

Diastase can be demonstrated in the spinal fluid in all cases, whether normal or otherwise, but the amount is usually small and the observed variations not significant; the amount is independent of the cell count (Leschke and Pincussohn)<sup>118</sup>.

*Antibodies* pass from the serum into the cerebrospinal fluid only in minimal amounts or not at all, except when inflammatory exudation occurs, and even then the antibody concentration is usually low.<sup>119</sup> Even simple chemicals enter the normal spinal fluid but very little,<sup>120</sup> although in tuberculous meningitis iodides and bromides given by mouth are more likely to enter the fluid than in non-meningitic diseases.

Dochez<sup>121</sup> reports that the spinal fluid is unique among serous cavity fluids in that it contains normally neither proteolytic

<sup>116</sup> Deut. Arch. klin. Med., 1913 (113), 92.

<sup>117</sup> Jour. Amer. Med. Assoc., 1920 (74), 1310.

<sup>118</sup> Deut. med. Woch., 1917 (43), 8.

<sup>119</sup> Lemaire and Debré, Jour. physiol. et path. gén., 1911 (13), 233; see also Lindig, Deut. med. Woch., 1918 (19), 430.

<sup>120</sup> See Rotky, Zeit. klin. Med., 1912 (75), 494.

<sup>121</sup> Jour. Exp. Med., 1909 (11), 718.

enzymes nor antienzymes. In the blood both are present, the antienzymes greatly predominating so that proteolysis does not occur. In pathological conditions both enzymes and antienzymes may make their appearance in the exudates in the spinal fluid. In acute meningitis, enzymes and antienzymes are present, and with pneumococcus and streptococcus infection the enzymes may predominate. With non-inflammatory transudates the antienzyme only is found, but in tuberculous meningitis the fluid occupies an intermediate position, usually exhibiting distinct antienzymatic action upon the proteolytic enzymes or leukocytes. The antienzymatic action is associated with a serum albumin and in inflammations of the pleura and peritoneum the amount of antienzyme usually preponderates over the proteolytic enzymes present.

The permeability of the meninges is increased in tuberculous meningitis even more than in suppurative meningitis, not only for crystalloids but also for the larger molecular complexes that constitute immune bodies. Thus, the normal anti-sheep corpuscle amboceptor of human blood is demonstrable in the spinal fluid in tuberculous meningitis, and also complement.<sup>122</sup>

Presumably certain amounts of a tuberculin-like material may be present in the spinal fluid in tuberculous meningitis, for positive skin tests have been obtained with such fluid<sup>123</sup> injected into persons sensitive to tuberculin.

<sup>122</sup> Weil and Kafka, *Wien. klin. Woch.*, 1911 (24), 335.

<sup>123</sup> Korbsch, *Med. Klinik*, 1921 (17), 816; Nasso, *Pediatria*, 1922 (30), 193.

## CHAPTER X

### CHEMISTRY OF THE SPUTUM<sup>1</sup>

Ott introduces this topic with the following statement, which holds good to this day:

The chemical investigation of the sputum has up to the present time, in contrast to the microscopic investigation, produced very little scientifically and practically valuable results. The basis of this is in part the impossibility of obtaining the secretion of the diseased parts of the lung in pure condition. The sputum, as it is delivered to us by the patient, always contains a considerable quantity of foreign admixtures, secretion of the bronchial tree, of the nasal and pharyngeal cavities and especially saliva; we are not in a position to separate more than approximately these undesired additions from the true secretion. Also we lack a usable object of comparison, for the secretion of the normal respiratory apparatus, which might serve as such, on account of its trifling amount has not yet been the subject of chemical investigation.

The sputum in tuberculosis has chemically nothing distinctive, since it may at times partake of the character of coexisting bronchiectasis, of gangrenous foci, or of an acute complicating pneumonia. The general chemical features of sputum, as far as can be determined in its more or less saliva-contaminated condition, are as follows: The basis is a weak solution of mucin, 0.5 to 3 per cent in strength, to which has been added in varying proportions the constituents of the blood, migrated and desquamated cells, products of autolytic and bacterial disintegration of the bronchial contents, and secretions of the buccal cavity.

#### MUCIN

As the chief and most characteristic constituent of the sputum, the chemistry of mucin may be appropriately discussed.

The name mucin is applied to a group of glyco-proteins found widely distributed in nature, characterized physically by their stringy mucilaginous

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<sup>1</sup> In the preparation of this section we have made much use of the splendid and exhaustive monograph "Das Sputum" by Heinrich von Hoesslin, Berlin, 1921, Julius Springer, publ.

character, and chemically by having the protein united to a complex radical, containing sulphuric acid and a carbohydrate. It is usual to differentiate mucins and mucoids, the latter differing in not being precipitated from weak alkaline solutions by slightly acidifying, and including tendomucoid, ovomucoid of eggs, osseomucoid, etc. Pseudo-mucins are distinguished by being alkaline in reaction, and not precipitating with weak acetic acid. Paramucin is the name given to the mucoids which yield reducing substances without previous decomposition.

The mucin of the bronchi and trachea resembles that obtained from other mucin-secreting glands and is probably not a little different from the glycoproteins found in tendons and connective tissues. Because of the acid radical, mucin is distinctly acid in character, probably dissolving only as its salts. It is perhaps because of this acid character that mucin exhibits the strong affinity for basic dyes, such as alum-hematoxylin, which is so strikingly seen in histological preparations. The general similarity of mucins in elementary composition, as well as the existence of distinct differences, is shown by the following table:<sup>2</sup>

|                        | C     | H    | N     | S         | O     | AUTHOR              |
|------------------------|-------|------|-------|-----------|-------|---------------------|
| Snail's mucin.....     | 50.32 | 6.84 | 13.65 | 1.75      | 27.44 | Hammarsten          |
| Tendon mucoid.....     | 48.76 | 6.53 | 11.75 | 2.33      | 30.63 | Chittenden and Gies |
| Submaxillary mucin ... | 48.84 | 6.80 | 12.32 | 0.84      | 31.30 |                     |
| Salivary mucin.....    | 48.26 | 6.91 | 10.70 | 1.38-1.45 |       | Müller              |

Apparently the non-protein radical of the several mucins is quite similar to that in the glycoprotein of cartilage, chondrin, and perhaps also in amyloid. The studies of Levene<sup>3</sup> indicate that the non-protein radicals of mucins are of two sorts: One, chondroitin-sulphuric acid, contains the nitrogenous hexose, chondrosamine, isomeric with glucosamine, and is found in cartilage, tendons, aorta and sclera; the other, mucoitin-sulphuric acid, has as its carbohydrate chitosamine, and is found in gastric and umbilical cord mucin, vitreous humor, cornea and ovarian cysts. Presumably the mucin of sputum belongs to the latter class.

The protein component of various mucins and mucoids probably differs. Elliott<sup>4</sup> found that the protein of mucin is capable of acting as an antigen, the antiserum produced reacting not only with the mucin used in producing it, but also, although less strongly, with the mucins from other species of animals, indicating the probable existence of a chemical relationship in the protein element of the mucins of different species, although not an identity. As yet we have no chemical analyses of the proteins of the various mucins, chiefly because of the difficulty and uncertainty of their purification, for

<sup>2</sup> From Mathew's Physiological Chemistry.

<sup>3</sup> Jour. Biol. Chem., 1918 (36), 105.

<sup>4</sup> Jour. Infect. Dis., 1914 (15), 501.

they occur mixed with other proteins, some of which, e.g., nucleoproteins, have very similar solubilities. Therefore we do not know whether the mucin secreted by the bronchial mucosa is chemically different from that produced in the salivary glands, or in the stomach. It is probable that it is different from the mucin of the tendons and of the connective tissues, which is so abundant in foetal life as seen in the Wharton's jelly of the umbilical cord. However, the immunological studies of Elliott indicate that there is some similarity between the protein components of salivary mucin and tendon mucin.

Fr. Müller found that sputum mucin contained 36.9 per cent of reducing substance while in submaxillary mucin he found but 23.5 per cent, but it is doubtful if these figures are constant and significant. He did show that sputum mucin contains a carbohydrate group resembling glucosamine. Maliwa<sup>5</sup> found that in autolysis of sputum the mucin undergoes disintegration rapidly, with liberation of the carbohydrate radical.

Recognizing the limitations of accuracy possible in the chemical study of sputum, we present the following summary of existing information on this subject:

### *1. Specific gravity*

Kossel<sup>6</sup> found that this varied directly with the amount of pus, and so no typical figures were found for different diseases. His actual figures were as follows:

|                             | AVERAGE | MAXIMUM | MINIMUM |
|-----------------------------|---------|---------|---------|
| Bronchitis.....             | 1008.3  | 1014    | 1004.3  |
| Emphysema.....              | 1010.6  | 1013    | 1006.2  |
| Lung abscess.....           | 1016.7  | 1018    | 1015.5  |
| Pulmonary tuberculosis..... | 1012.9  | 1026    | 1008.0  |
| Pneumonia.....              | 1013.9  | 1020    | 1010.4  |

### *2. Reaction*

Usually this is faintly alkaline, but sometimes decomposition of sputum contained in cavities leads to sufficient production of such organic acids as formic, acetic and butyric, and perhaps others, to give a neutral or slightly acid reaction. On the other hand, the decomposition may result in an excessive production of ammonia, leading to the formation of a sputum which may be excessively alkaline when first expectorated, but becoming rapidly

<sup>5</sup> Deut. Arch. klin. Med., 1914 (115), 407.

<sup>6</sup> Zeit. klin. Med., 1888 (13), 149.

less alkaline and sometimes even acid. This is especially likely to be the case if there is gangrene. Bück found that in tuberculosis the fresh sputum is neutral to litmus, becoming later distinctly acid; and Maggiorano found the reaction always acid in advanced phthisis (von Hoesslin). The usual alkalinity of sputum is attributed in part to free alkali carbonate, and in part to alkali salts of the mucin, which is itself acid (F. Müller). If there is much admixture of plasma with the sputum the reaction will tend to approach that of the blood.

### 3. Inorganic constituents

These correspond closely to those of the blood, except for the addition of inhaled dust particles, and rarely salts are precipitated in stagnated fluid collections. As is the case with other secretions and exudates, by virtue of their diffusibility the inorganic elements vary less, and also are more like those of the blood, than the organic components. This is shown by the following table from von Hoesslin:

| CHARACTER OF THE SPUTUM                                | AUTHOR    | FRESH SUBSTANCE |               | COMPO-NENTS FRESH SUBSTANCE |          | COMPONENTS DRY SUBSTANCE |          |
|--|-----------|-----------------|---------------|-----------------------------|----------|--------------------------|----------|
|  |           | Water           | Dry substance | Inor-ganic                  | Or-ganic | Inor-ganic               | Or-ganic |
|  |           | per cent        | per cent      | per cent                    | per cent | per cent                 | per cent |
| Bronchitis, muco-purulent...                           | Biermer   | 97.90           | 2.10          | 0.55                        | 1.55     | 26.23                    | 73.77    |
| Bronchitis, mucous.....                                | Wright    | 95.60           | 4.40          | 0.50                        | 3.90     | 11.36                    | 88.64    |
| Bronchitis, mucous.....                                | Simon     | 94.17           | 5.83          |                             |          |                          |          |
| Chronic bronchitis, muco-purulent.....                 | Bamberger | 95.62           | 4.38          | 0.67                        | 3.71     | 15.30                    | 84.70    |
| Bronchitis, mucous.....                                | Renk      | 97.67           | 2.33          | 0.65                        | 1.68     | 28.43                    | 71.57    |
| Bronchiectasis, purulent .....                         | Bamberger | 93.86           | 6.14          | 0.79                        | 5.35     | 12.87                    | 87.13    |
| Bronchiectasis.....                                    | Falk      | 94.66           | 5.34          | 0.67                        | 4.67     | 12.55                    | 87.45    |
| Tuberculosis, purulent .....                           | Bamberger | 93.47           | 6.53          | 0.76                        | 5.77     | 13.17                    | 86.83    |
| Tuberculosis, purulent, with some blood.....           | Biermer   | 90.70           | 9.30          | 0.87                        | 8.43     | 11.84                    | 88.52    |
| Tuberculosis.....                                      | Renk      | 94.46           | 5.54          | 0.82                        | 4.72     | 14.96                    | 85.04    |
| Pneumonia before the crisis, rusty brown.....          | Biermer   | 92.66           | 7.34          | 0.77                        | 6.57     | 12.19                    | 87.81    |
| Pneumonia after the crisis, decolored, muco-purulent.. | Biermer   | 89.03           | 10.97         | 1.58                        | 9.39     | 14.36                    | 85.64    |
| Pneumonia, fatal, jelly-like..                         | Bamberger | 94.19           | 5.81          | 1.02                        | 4.79     | 21.29                    | 78.71    |

Bamberger published in 1861 the following table of determinations of the inorganic salts of sputum:

In 100 parts of inorganic salts were contained:

|   | BRON-CHITIS | BRON-CHI-ECTASIS | CHRONIC TUBER-CULOSIS | TUBER-CULOUS PNEU-MONIA | PNEUMONIA    |             |
|---|-------------|------------------|-----------------------|-------------------------|--------------|-------------|
|   |             |                  |                       |                         | Early stages | Reso-lution |
| Chlorine.....                               | 40.764      | 35.033           | 35.775                | 33.395                  | 37.445       | 47.211      |
| Sulphuric acid.....                         | 1.246       | 1.611            | 0.701                 | 0.801                   | 8.371        | 2.617       |
| Phosphoric acid.....                        | 10.080      | 13.120           | 13.048                | 14.153                  | Traces       | 1.034       |
| Potassium.....                              | 16.163      | 22.496           | 24.066                | 19.986                  | 41.198       | 14.634      |
| Sodium.....                                 | 36.000      | 30.122           | 27.904                | 31.686                  | 14.970       | 37.235      |
| Calcium phosphate.....                      | 2.437       | 1.534            | 1.627                 | 4.322*                  | 2.108        | 3.961       |
| Iron phosphate.....                         | 0.093       | 0.440            | 0.090                 | 0.141                   | 1.028        | 0.422       |
| Magnesium phosphate.....                    | Traces      | 1.006            | 1.204                 |                         |              |             |
| Ca and Mg as carbonate<br>and sulphate..... | 0.475       | 0.954            | 1.743                 | 0.218                   | 1.331        | 0.886       |
| Silicic acid.....                           | 1.036       | 0.116            | 0.900                 | 0.300                   | 0.630        | 0.181       |

\* Includes magnesium.

No later analyses by more modern methods seem to have been reported, and in view of the antiquity of these figures, and the probable admixture of saliva with the sputum, their present value is open to question. In all probability the inorganic constituents of sputum are generally much more nearly uniform than Bamberger found. Possibly extensive disintegration of nuclear material in the sputum may increase the amount of phosphates slightly, while hemorrhage will increase the iron and potassium. Kossel found, accordingly, higher figures for both organic and inorganic phosphorus in the sputum in tuberculosis than in pneumonia, and Falk<sup>7</sup> found about 30 per cent of the phosphorus in purulent tuberculosis sputum soluble in lipoid solvents.

The following figures for the CaO content of sputum (in parts per thousand of fresh weight) are given by Loeper and Béchamp:<sup>8</sup> Pneumonia, 0.46; bronchopneumonia, 0.50, 0.70; advanced phthisis, 0.30, 0.38, 0.56, 0.90; bronchitis, 0.36, 0.24. These figures may be compared with those for exudates, 0.18 to 0.25.

<sup>7</sup> Ergeb. Physiol., 1910 (9), 406.

<sup>8</sup> Compt. Rend. Soc. Biol., 1910 (69), 178.

Crystals of calcium oxalate may sometimes be found in the sputum.<sup>9</sup>

Ammonio-magnesium phosphate crystals may be deposited when alkaline reaction from ammonia formation occurs, as in gangrene, abscesses, bronchiectasis and tuberculous cavities. They appear in the well known "coffin lid" form. Possibly calcium phosphate crystals may be formed in similar lesions, or fragments of disintegrated calcified tuberculous lesions may sometimes be found in the sputum.

As von Hoesslin says:

By and large, the estimation of the separate inorganic constituents of sputum is of relatively little value, for no typical differences are shown, except for Na, K and P in pneumonia. Bronchitis sputum shows a somewhat higher water content, and perhaps also higher total ash.

#### *4. Proteins*

These vary much more than the inorganic constituents, both quantitatively and qualitatively. In general, the simplest composition would approximate that of a weak solution of mucin, with traces of nucleoprotein and plasma proteins; presumably the composition of the normal secretion of the bronchi would be this if it could be collected in sufficient quantities for analysis. With inflammation comes an addition of blood proteins, increasing in proportion with the amount of exudation, until in acute pneumonia the mucin is completely overshadowed. The pouring out of leukocytes adds to the proportion of nucleoproteins, while their proteolytic activity adds varying quantities of cleavage products: i.e., proteoses, peptones, amino acids and purines, together with small quantities of fats and fatty acids. The stimulated activity of the mucus secreting glands in simple bronchitis, on the other hand, would produce a sputum consisting chiefly of mucin. Obviously in tuberculosis we may find in different stages and types of the disease any and all possible variations in the composition of the sputum, from that of a simple bronchitis, to the pus of a

<sup>9</sup> Hoesslin credits the statement of Kaatzer that he found large quantities of ammonium oxalate in a tuberculous cavity, but as the original article is not accessible we cannot verify this improbable report.

freshly emptying abscess, or the foul disintegrating material of a gangrenous focus. The same gamut of changes may occur from day to day in the sputum of the same patient.

There seems to be nothing particularly significant in the proportion of *mucin* in the sputum. Probably the best analyses are those of Wanner,<sup>11</sup> who determined the amount of reducing substance obtained by hydrolyzing the sputum with 10 per cent HCl, and calculated the mucin on the assumption of its yielding 33.6 per cent by weight of carbohydrate, and that under these conditions no appreciable amounts of carbohydrates come from the nucleoproteins. His results are as follows, in per cent of the moist weight calculated as mucin:

|                 |             | AVERAGE |
|-----------------|-------------|---------|
| Bronchitis..... | 1.024-3.310 | 2.088   |
| Phthisis.....   | 0.735-0.789 | 0.760   |
| Pneumonia.....  | 0.660-1.030 | 0.930   |

In all these conditions, however, the mucin is much the most abundant protein, if Wanner's figures are correct.

After removal of the mucin by precipitation with acetic acid, which presumably takes down also nucleoproteins, cells and perhaps other things, a certain amount of coagulable protein and non-coagulable nitrogen remains in solution. Wanner gives the following average figures (in 100 parts of fresh sputum) for these components in sputum from various conditions:

|                                | COAGU-LABLE PROTEIN | ALBU-MOSES | ALBUMOSEN | RESIDUAL-N |
|--------------------------------|---------------------|------------|-----------|------------|
| Chronic bronchitis.....        | Traces              | 0.319      | 0.050     | 0.100      |
| Bronchopneumonia (1 case)..... | 0.364               |            |           |            |
| Bronchiectasis.....            | 0.362               | 0.348      | 0.056     | 0.198      |
| Tuberculosis.....              | 0.503               | 0.320      | 0.051     | 0.152      |
| Infarct.....                   | Traces              | 0.025      |           |            |
| Gangrene (1 case).....         | 0.327               | 0.264      | 0.049     | 0.286      |
| Pneumonia.....                 | 1.432               | 0.462      | 0.073     | 0.208      |

Practically the presence of sufficient *coagulable protein*, to be readily detected by simple methods, is seen only when there is

acute inflammation,<sup>10</sup> and hence is looked upon as of diagnostic significance. As seen from the above table Wanner<sup>11</sup> found characteristic variations in the amount of coagulable protein in sputum from different conditions, as follows: In bronchitis the amount is very small; in bronchiectasis coagulable protein is present, but the amount of uncoagulable nitrogen (due to autolysis) is relatively large; in phthisis as well as in bronchiectasis the amount of this protein does not exceed 1 per cent; in pneumonia it may reach 3 per cent but it is highest in pulmonary gangrene. Any protein content that causes more than a slight turbidity on boiling indicates an inflammation.<sup>12</sup> Albumin, or better, coagulable protein is also present in the sputum of patients with pulmonary edema and pleurisy. According to Works,<sup>13</sup> in active tuberculosis there is usually 0.2 per cent or more of coagulable protein in the sputum.

Takeuchi<sup>14</sup> found that when tubercle bacilli are present in the sputum, lymphocytes, eosinophiles and coagulable protein will be found, but in the absence of tubercle bacilli none of these three elements is present.

It is evident from the above facts that the presence or absence of coagulable protein in the sputum can have no positive diagnostic value, although it may have some significance. It is equally true that the amount of coagulable protein does not indicate the stage of the disease, although it is safe to say that there is usually less protein in the sputum in early and in healing cases than during active advanced stages. Wanner found that only tuberculous sputum of homogeneous and fluid consistency contains

<sup>10</sup> H. Roger (Compt. Rend. Soc. Biol., 1913 (75), 103) believed that the coagulable protein of sputum must come from the tissues, since it lowers blood pressure, but there are so many other elements present in this fraction of the sputum that the depressor effect cannot be considered as proof of the origin of the protein.

<sup>11</sup> Deut. Arch. klin. Med., 1903 (75), 347.

<sup>12</sup> It is to be considered that the amount of protein is greater in the solid portions of the sputum, and the more thoroughly the sputum is agitated before filtration the more coagulable protein will be found. (Hempel-Jørgensen, Beitr. Klin. Tuberk., 1913 (26), 392.)

<sup>13</sup> Jour. Amer. Med. Assoc., 1912 (59), 1537.

<sup>14</sup> New York Med. Jour., 1921 (113), 574.

regularly a relatively large amount of coagulable protein. Perhaps the chief value of protein determination lies in differentiating bronchitis from edema, in which the amount of coagulable protein is high. Of course the occurrence of hemorrhage or of a complicating edema will raise the amount in tuberculous sputum. Cocke<sup>15</sup> reviewed reports of examinations in 3951 cases of tuberculosis in which there was a positive test in 95.6 per cent, and from this material and his own experience, drew the following conclusions:

1. All cases of pulmonary tuberculosis showing bacilli give a positive albumin reaction in the sputum.
2. Frequently albumin will be found in the sputum in incipient tuberculosis before bacilli are present, but its presence is variable and it cannot be relied upon as a definite means of diagnosis in incipient cases.
3. The amount of albumin present in the sputum in tuberculosis depends upon the extent of the exudative or destructive process in the lungs, is always associated with an alveolitis, and may be an index of the activity of the process, the heavier the albumin content the more activity present.
4. Certain (fibrosing or proliferating) forms of definite tuberculosis fail to show an albumin reaction in the sputum. Cases cured for two years or more will not show it.
5. When physical signs and the tuberculin reaction fail to show activity the presence of albumin in the sputum may do so.
6. As a diagnostic means its value is limited and relative and the result of a single examination misleading. It perhaps has greater value prognostically, as above shown.

We have no reliable figures as to the proportion of serum albumin and globulins in the coagulable proteins found in sputum, but according to A. Oswald,<sup>16</sup> serum albumin and pseudoglobulin pass through the vessel walls more readily than euglobulin and fibrinoglobulin, and hence the two latter proteins would probably predominate only in very acute exudative inflammatory lesions.

Immediately after tapping a chest for pleural effusions, there sometimes occurs a sudden outpouring from the bronchi of a thin

<sup>15</sup> Amer. Jour. Med. Sci., 1914 (148), 724.

<sup>16</sup> Zeit. exp. Path., 1910 (8), 226.

serous fluid, which contains much coagulable protein. Probably it represents an acute edema resulting from the sudden circulatory change within the decompressed lung. Ott gives tables showing the presence of 3 to 5 per cent protein in most of the specimens of such sputum.

*Nucleoproteins* are always present in sputum, in direct proportion to the number of cells. In proportion to the amount of cellular disintegration they may be found free, and much of the viscosity of the sputum in late pneumonia depends on the large amount of free nucleoprotein which is even more sticky and tenacious than mucin. Analyses show that such sputum does not possess so much mucin as the sputum of simple bronchitis. H. Kossel<sup>6</sup> determined the protein phosphorus in sputum, and assuming 3.5 per cent of phosphorus in nucleoproteins, found in tuberculosis from 0.100 to 0.334 gram per day (average 0.243 gram); and in pneumonia, 0.060 to 0.298 gram (average 0.170 gram), but as in tuberculosis from 5 to 10 times as much sputum was secreted, the proportion of nucleoprotein was higher in pneumonia. Simon<sup>17</sup> was unable to find either nucleo-histon or histon in tuberculosis sputum.

##### 5. Cleavage products of the proteins

A certain amount of digestion of the sputum may take place while it is still in the lung, and some of the unabsorbed cleavage products may be present in the expectorated sputum. Except in pulmonary gangrene (when much of the disintegration is produced by bacteria), leucocytic enzymes are chiefly responsible for whatever proteolysis occurs, and hence the amount of cleavage products will depend on the number of polymorphonuclear leucocytes and the length of time the material stagnates in the lungs. A purulent sputum from a cavity will contain the largest proportion of cleavage products, except the material from an active area of gangrene.

Proteolytic ferments may be demonstrated in the sputum, if it contains a sufficient quantity of leucocytes, and hence the strongest ferment test reactions are obtained in pneumonia, while in tuberculosis no positive tests can usually be obtained except when the

<sup>17</sup> Arch. exp. Path. Pharm., 1903 (49), 449.

sputum is made purulent by some acute inflammatory complication. The method usually employed for this purpose is that of Müller and Jochmann: A drop of the faintly alkalinized sputum is placed on a plate of coagulated blood serum in a Petri-dish, and this is incubated at 55°. If proteolytic ferments active in an alkaline medium (leucoprotease) are present there will be found after twenty-four to forty-eight hours a distinct depression in the coagulum, bacterial action being prevented by the high temperature.

Tuberculous sputum contains tuberculin, or a substance giving tuberculin tests on intracutaneous injection in reactive subjects.<sup>17a</sup>

“Albumose.” Presumably all possible products of proteolysis are present in the sputum, but ordinarily the quantities present are too small to permit of their demonstration in the amounts of sputum that are available. In the table of Wanner on page 246 we have given his figures which show that substances giving the precipitation reactions of albumoses are present in sputum in considerable amounts, but it is certain that by no means all of the material so designated by Wanner is albumose; probably it represents a mixture of incompletely separated mucin and serum proteins together with cleavage products. However, Eiselt<sup>18</sup> also found oftentimes in the sputum more albumose-like material than coagulable protein, the amount fluctuating greatly. Of course still higher figures for “albumose” are usually found in pneumonia after the crisis. Eiselt sometimes found a small amount of precipitable nitrogen in the filtrate from his albumose fraction, which he interprets as peptone, but the nature of this material was not positively established.

Simon<sup>17</sup> found considerable “albumose” in phthisical sputum as well as lower cleavage products. Prorok<sup>19</sup> agrees with Eiselt that in some cases of tuberculosis the amount of albumose may exceed the coagulable protein, which rarely is more than 1 per cent of the moist weight. Staffregen could find no true peptone in phthisical sputum, but Stadelmann<sup>20</sup> found that such sputum con-

<sup>17a</sup> Maniscalco, *Tuberculosis*, 1922 (14), 1.

<sup>18</sup> *Zeit. klin. Med.*, 1912 (75), 71.

<sup>19</sup> *Munch. med. Woch.*, 1909 (56), 2053.

<sup>20</sup> *Zeit. klin. Med.*, 1889 (16), 128.

tained enzymes hydrolyzing fibrin, and attributed this largely to bacteria, but probably most of the enzymes present in sputum come from the leucocytes. In the early stage of pneumonia the sputum has no proteolytic action, presumably because inhibited by the large amount of serum present; but with resolution active proteolytic properties appear (Bittorf).<sup>21</sup> Pneumonic sputum before the crisis has but slight action on peptids, but acquires marked peptolytic activity thereafter.<sup>22</sup>

*Amino acids* (leucine and tyrosine) have been found free in sputum, especially in bronchiectasis and abscesses, but they probably are not ordinarily present in recognizable quantities in tuberculous sputum. The Millon test for the presence of free tyrosine is said to be negative in tuberculosis sputum, although often positive with pneumonic sputum, but not constantly enough to make this a valuable diagnostic method with sputum as it is said to be with pus.<sup>23</sup> Eiselt,<sup>18</sup> however, states that in sputum from 45 cases of phthisis, he was able to demonstrate by the copper salt method, in 10 free leucine, in 8 tyrosine and in 2 alanine. Fischer<sup>24</sup> reported finding free leucine crystals in the fresh sputum of a tuberculous gangrene, tyrosine being missing.

Pissavy and Monceaux<sup>25</sup> obtained reactions for tyrosine in all of 183 specimens of sputum containing tubercle bacilli, and in no cases of recovered tuberculosis, but the tyrosine test was positive in pulmonary gangrene and lung abscess. They state that positive tyrosine reactions depend on destruction of tissue and not on the presence of leucocytes. Although tyrosine was demonstrated directly in some of their cases, the usual test was the browning of the deproteinized sputum by extracts of *Russula delica*, a reaction which is possibly not so specific for tyrosine as these authors assume.

Neither peptones nor polypeptides have been certainly found in tuberculosis sputum, but their identification is probably only a

<sup>21</sup> Deut. Arch. klin. Med., 1907 (91), 212.

<sup>22</sup> Abderhalden, Zeit. physiol. Chem., 1912 (78), 344.

<sup>23</sup> See Salmoni, Gazz. degli. Osped., 1908 (29), 1381; Nicola, Riv. d'igiene e san. pubbl., 1911 (22), 296.

<sup>24</sup> Maly's Jahresber., 1879 (9), 361.

<sup>25</sup> Bull. Soc. Med. d. Hôp., 1922 (38), 376.

question of sufficient material and care. All investigators of the question recognize the presence of considerable amounts of residual-N after removal of proteins and proteoses (see Wanner's table, page 246), and this undoubtedly consists largely of amino acids and peptids, besides purines from the nucleoproteins and diffusible nitrogenous constituents from the blood. Presumably glucosamine from the digested mucin is also present.

In hemoptysis there is a tendency for the coagulation of the escaped blood to be slow or absent, which Maliwa<sup>5</sup> attributes at least in part to the anticoagulating action of the protein cleavage products that become mixed with it in the lung.

*Urea* must be present in the sputum in much the same proportion as in the blood, since, because of its very great diffusibility, it is found uniformly distributed throughout all the fluids of the body, and in uremia large amounts have been found in the sputum.

Probably in tuberculosis sputum the daily variation in *residual-N* in the sputum gives an excellent index of the amount of autolysis of exudate and tissues that is going on within those parts the lung that are producing the sputum. The variations are therefore large between different cases even in the same stage, and from day to day in the same case, but of course the highest figures will be obtained in actively progressing cases with much suppuration or tissue destruction, that drain freely into the bronchi. Sputum may contain indole, derived either from the putrefying proteins or excreted from the blood.<sup>26</sup>

Xanthine and hypoxanthine are formed in the autolyzing lung (Long and Wells)<sup>27</sup> and hence are undoubtedly present in traces in the sputum, but probably if any uric acid is present it represents diffusion from the blood, as with urea and creatinine. Von Hoesslin says that uric acid has never been demonstrated in sputum, but probably modern micro methods would show it.

Free ammonia and hydrogen sulphide have been described in the sputum in pulmonary gangrene and fetid bronchitis or bronchiectasis, and hence may be present when these putrefactive conditions complicate tuberculosis, but otherwise they are absent

<sup>26</sup> Binda and Cassarini, Gaz. Med. Ital., 1913 (64), 461; Cantelli, Riforma Med., 1922 (38), 481.

<sup>27</sup> Deut. Arch. klin. Med., 1914 (115), 377.

from tuberculosis sputum. Crystals of ammonio-magnesium phosphate may be found in the sputum under the same conditions. Among other substances described in gangrene sputum, and hence perhaps sometimes to be found in tuberculosis, are methylamine, phenol, cresol, volatile fatty acids, indole and skatole, cadaverin (pentamethylendiamine).

#### *6. Enzymes of sputum*

As far as known these are derived entirely from the cellular constituents, and therefore chiefly correspond to the enzymes of the leucocytes, which are discussed under the subject of autolysis in tuberculous areas (Chap. V). Maliwa<sup>5</sup> has studied the enzymatic activities of sputum in some detail and he determined the proteolytic activity with casein as a test object, preferring it to the plate method of Müller-Jochmann (see page 128) as more certain to exclude bacteria. He found it practically always possible to demonstrate in sputum the presence of a trypsin-like enzyme, digesting casein only in alkaline solution, and the activity varies in direct proportion to the number of leucocytes, except that when blood is present its antitryptic activity somewhat inhibits the enzyme. As the casein-digesting enzyme solution used by Maliwa did not split either silk peptone or glycyl-tryptophan, he believed that it is not the same as pancreatic trypsin.

Sputum that has been left standing, whether within the lungs or outside the body, shows for a time increasing proteolytic activity probably from liberation of enzyme from the disintegrating cells, and perhaps partly from destruction of the antienzymes. The sputum enzymes are also able to digest the mucin, this taking place fairly rapidly when sputum is allowed to autolyze.

Eiselt<sup>18</sup> emphasizes the marked variation in the proteolytic activity from day to day. This may be accounted for by the variations in the number of leucocytes on the one hand, and of the inhibiting antienzymes from blood and plasma. The latter were found to fluctuate greatly, usually being highest in the febrile period so that the presence of tryptase may then be entirely masked.

Sputum also contains enzymes that disintegrate polypeptides (peptidase) which may be readily demonstrated by incubating

sputum with glycyl-tryptophan; the liberated tryptophan can soon be demonstrated by the color test.

Apparently *lipases* are present in sputum, and this should be especially the case in tuberculosis since the lymphocytes are said to be particularly rich in fat-splitting enzymes. Although Eiselt was unable to demonstrate the presence of an enzyme splitting lecithin, Maliwa<sup>5</sup> found that in autolysis of sputum the amount of ether-soluble material increased, from liberation of the intracellular fat with an increase in the fatty acids. By antiseptic digestion of olive oil and sesame oil he demonstrated the presence of lipase in the sputum. The presence of peroxidase in tuberculous sputum was also shown by the guaiac test, but the salol-splitting enzyme was found only inconstantly in tuberculosis.

### *7. Lipins (fats, fatty acids and lipoids)*

While even the simplest catarrhal sputum contains a little fat, the amount increases in direct proportion to the number of cells that have entered the sputum, and some may come from the plasma lipoids when there is serous effusion. If the sputum stagnates there may be a further increase from disintegrated cells, since the other cleavage products are more readily absorbed from the cavity walls.

The following table by Bokay (taken from Ott) gives the proportion of the organic constituents of sputum in parts per thousand:

|                         | BRON-CHITIS IN TYPHOID | FIBROID PHTHISIS | PHTHI-SIS, EARLY IN APEX | PHTHI-SIS, CAVITIES | PHTHI-SIS, ADVANCED | PHTHI-SIS, ADVANCED |
|-------------------------|------------------------|------------------|--------------------------|---------------------|---------------------|---------------------|
| Fatty acids in fat..... | 0.224                  | 0.845            | 0.462                    | 2.468               | 3.468               | 9.725               |
| Free fatty acids.....   | Traces                 | 0.184            | 0.521                    | 0.370               | 0.307               | 0.902               |
| Soaps.....              | Traces                 | 0.380            | 0.430                    | 0.537               | 0.516               | 3.973               |
| Cholesterol.....        | Traces                 | 0.4              | 1.617                    | 0.172               | 1.160               | 0.141               |
| Lecithin.....           | Traces                 | Traces           | 1.543                    |                     | 1.165               | 1.245               |
| Nuclein.....            | Traces                 | 0.102            |                          |                     | 0.260               | 0.489               |
| Protein.....            | 0.898                  | 2.040            |                          | 4.430               | 3.455               | 5.115               |

v. Hoesslin<sup>1</sup> gives the following table:

| VARIETY OF SPUTUM                      | AUTHOR    | TOTAL FAT                |                        | AVERAGE DAILY AMOUNT<br>grams | NEUTRAL FAT | In per cent of ether extract | SOAPs | FATTY ACIDS |
|--|-----------|--------------------------|------------------------|-------------------------------|-------------|------------------------------|-------|-------------|
|  |           | Fresh weight<br>per cent | Dry weight<br>per cent |                               |             |                              |       |             |
|  |           |                          |                        |                               |             |                              |       |             |
| Mucous, pharyngeal.....                | Nasse     | 0.289                    | 6.497                  |                               |             |                              |       |             |
| Chronic bronchitis.....                | Jakobsohn | 0.103                    | 1.697                  | 0.073                         |             |                              |       |             |
| Mucopurulent tuberculo-sis.....        | Plesch    | 1.39                     | 15.16                  | 3.23                          |             |                              |       |             |
| Bronchiectasis.....                    | Falk      | 0.795                    | 15.27                  |                               |             |                              |       |             |
| Purulent mucous, acute bronchitis..... | Jakobsohn | 0.464                    | 4.219                  | 0.037                         |             |                              |       |             |
| Fetid bronchitis.....                  | Jakobsohn | 0.341                    | 7.141                  | 0.336                         | 60.75       | 20.08                        | 19.17 |             |
| Purulent tuberculosis ....             | Jakobsohn | 0.427                    | 5.828                  | 0.643                         |             |                              |       |             |
| Florid tuberculosis.....               | Jakobsohn | 0.489                    | 6.698                  | 0.595                         | 69.45       | 14.76                        | 15.79 |             |
| Empyema.....                           | Jakobsohn | 1.143                    | 13.317                 | 3.414                         |             |                              |       |             |
| Tuberculosis.....                      | Bück      | 0.611                    |                        | 0.544                         | 92.96       |                              | 7.04  |             |
| Tuberculosis.....                      | Renk      | 0.390                    | 7.010                  | 0.460                         |             |                              |       |             |
|  |           |                          |                        | 0.008                         |             |                              |       |             |
| Pneumonia.....                         | Jakobsohn | 0.121                    | 1.402                  | 0.016                         |             |                              |       |             |
| Pneumonia.....                         | Renk      | 0.026                    | 0.450                  |                               |             |                              |       |             |
| Serous edema.....                      | Jakobsohn | 0.270                    | 1.647                  | 0.059                         |             |                              |       |             |

As both the leucocytes and the lung tissue, as well as the bacteria, contain lipase, a certain amount of cleavage of the fats, and perhaps also of the lipoids, may take place in the sputum. As evidence of this we often find crystals of *fatty acids* in sputum, especially where it has stagnated for some time in cavities. They form a large part of the bronchial masses known as *Dittrich's plugs*. These crystals are characterized by being long and slender, sharply pointed, sometimes with bulgings, often bent, either free or in clusters, melting readily, soluble in fat solvents but not in water or weak acids.

Jakobsohn<sup>28</sup> gives as the melting point of the fatty acid mixture obtained by him in sputum, the following figures: Tuberculosis, 42.5°; fetid bronchitis, 41.0°. This indicates that in addition to palmitic acid (m.p., 62.2°) and stearic acid (m.p., 69.2°) there was

<sup>28</sup> Quoted by von Hoesslin,<sup>1</sup> p. 215.

probably also oleic acid (m.p. 14°). The amount of fatty acids and soaps in sputum is probably not large. Jakobsohn found in the ether extract of sputum in bronchitis foetida, 19.17 per cent free fatty acids and 20.08 per cent of soaps; in tuberculosis, 15.79 per cent fatty acids and 14.76 per cent of soaps. Bück found in tuberculous sputum 7.04 per cent of the ether extract as fatty acids and soaps.

*Cholesterol.* The cells in the sputum are often loaded with fatty granules, some of which may be doubly refractile. These are the myelin granules, which consist chiefly of a mixture of fats and cholesterol esters. The myelin cells seem to be mostly desquamated flat alveolar epithelial cells, and they are found especially abundant in the "morning sputum" of healthy persons, and hence their presence in tuberculosis sputum is of no significance, except that they are said to be less numerous in more advanced lesions. The cholesterol esters are readily hydrolyzed, and hence free cholesterol crystals may occasionally be found in the sputum from old cavities.

Cholesterol is, of course, to be extracted from sputum with ether, since it is present in every cell. Bueck found from 32 to 70 mgm. in the day's sputum of 4 cases of tuberculosis, the cholesterol constituting from 1.07 to 4.50 per cent of the entire ether extract. Jakobsohn<sup>28</sup> found in 2 cases of tuberculosis an average of 10.49 per cent of the ether extract to be cholesterol. We can find no reports of analyses of sputum by modern methods which differentiate free cholesterol from cholesterol esters.

*Phosphatids.* These, like the cholesterol, vary in amount with the number of cells, although probably they are destroyed in old pus whereas the cholesterol persists unchanged. We have only the following figures, cited by v. Hoesslin (p. 219): Jakobsohn calculated the phosphatids in two specimens of tuberculosis sputum as 10.4 and 16.8 per cent of the total ether-soluble material; Falk found in a specimen of bronchiectasis sputum, 2.08 per cent.

### *8. Carbohydrates*

Although the mucin and nucleoprotein of the sputum introduce much bound carbohydrate, free sugars have seldom been demonstrated in fresh sputum. The demonstration of traces of reducing substance in sputum ordinarily means nothing, since the mouth

saliva contains always more or less free sugar from the food or from salivary digestion of starches. Of course a trace of sugar may be expected in sputum since the blood normally contains 0.1 per cent. There are a few reports of the demonstration of reducing carbohydrates in the sputum of tuberculosis in diabetics, that are probably correct.

If fresh sputum is hydrolyzed a small amount of *glycogen* will be obtained, since the cells always contain more or less of this carbohydrate. If the cells are retained in the exudate until they disintegrate, the glycogen is destroyed, hence there is usually more sputum glycogen in acute than in chronic lesions. Nevertheless, although the pneumonic lung was found by Loeper to contain but 0.85 per cent of glycogen, Pozzilli<sup>29</sup> found as much as 2 to 3 per cent in tuberculosis sputum in advanced stages, 0.15 to 0.6 per cent in earlier stages, 0.25 per cent in fetid bronchitis, 0.05 per cent in pneumonia, and none in bronchial catarrh.

#### 9. *Pigments of sputum*

The yellowish or greenish color frequently observed depends generally, except in the case of icterus, upon blood pigment transformed by bacterial action, and perhaps also by cellular action. Even when no microscopically visible erythrocytes can be found in sputum, it will usually, if at all colored, give a good guaiac test, thus establishing the presence of constituents of hemolyzed corpuscles. Once the red cells escaping into the lung cavities undergo hemolysis, a series of pigments are formed. The hemoglobin itself probably does not remain long intact under the conditions present in tuberculous cavities, but soon splits into the protein (globin) and pigmentary (hematin) components. The hematin in turn splits into an iron-free pigment, hematoidin, and the iron-containing hemosiderin, either or both of which may be found in the sputum.

Hematoidin, being similar to although not identical with bilirubin, may produce a greenish-yellow coloration of the sputum. This is probably the chief pigment that imparts the greenish color seen at the site of absorbing subcutaneous hemorrhages. Usually

<sup>29</sup> *Gazetta degli Ospedali*, 1908 (29), No. 113.

it is present in solution, but rarely may occur as yellowish granular masses or even as rhombic crystals or clusters of needles. Probably it does not often form crystals in sputum as it sometimes does in large old blood clots, but crystals believed to be hematoidin have been described in tuberculosis sputum by a few observers, and v. Jaksch stated that they often appear after tuberculous hemoptysis.

*Hemosiderin* forms the brownish-yellow granules which are so characteristic a feature within the cells of the sputum expectorated in chronic cardiac decompensation ("heart failure cells") and less often in the sputum of tuberculosis. Von Hoesslin says,

The heart-failure cells are seldom found in tuberculosis, and are entirely missing in purulent sputum for here the cells quickly disintegrate and the pigment is dissolved. However, in fulminating cases Lewy has found them extraordinarily abundant. They were formerly not supposed to appear after hemoptysis, but undoubtedly they do occur, although one often wonders that they are not seen more often.

The chemical nature of hemosiderin is not known, but as it stains so readily for iron with reagents, it is believed to be either in a free inorganic form, perhaps a mixed oxide and carbonate of iron, or at most very loosely combined with organic radicals. Being relatively insoluble it is removed by the phagocytes and hence is found chiefly as an intracellular constituent of the tissues. If putrefaction is taking place in the lung with liberation of hydrogen sulphide, the hemosiderin may be converted into iron sulphide, forming in the tissues a black deposit (pseudomelanosis) but in the sputum the iron sulphide would resemble carbon particles.

Methemoglobin may also be formed in the sputum by the action of bacteria, both pneumococci and *Streptococcus viridans* producing this pigment as seen in the green color of their colonies on blood agar plates.<sup>30</sup> This may be a factor in coloring sputum when these organisms are present. When putrefaction is present we may get sulphur-methemoglobin as a green pigment in sputum, this being the pigment that is so commonly seen discoloring the abdominal wall in cadavers. This sulphur compound is readily decomposed by weak acids, even  $\text{H}_2\text{CO}_3$ , and then yields the equally greenish methemoglobin.

<sup>30</sup> See Cole, Jour. Exp. Med., 1914 (20), 363; Blake, ibid., 1916 (24), 315.

Bile pigments are present in the sputum in icterus, and may be demonstrable there when there is no visible jaundice. Presumably the bile salts are present whenever there is obstructive icterus.

Coal pigment and other inhaled pigments appear in the sputum in tuberculosis but are, of course, of no particular significance.

### 10. Charcot-Leyden crystals

These are rarely found in tuberculosis, located in the mucinous portions of the sputum, being more characteristic of bronchial asthma, as they seem to be the product of a chronic inflammation of the bronchial mucosa.

They were described by Charcot as colorless, refractile, elongated octahedra; insoluble in alcohol, ether, and glycerol; soluble in hot water, acids, and alkalies; size variable, from 0.016 by 0.005 mm. up. These crystals have been found not only in sputum, but also in the tissues and blood of cadavers, and occasionally in the freshly drawn blood of leukemics. They are characterized by resistance to putrefaction and autolysis of the sputum. Poehl<sup>31</sup> believed them to be the same as Böttcher's spermin crystals, and derived from decomposed nucleins. Schreiner considered that these spermin crystals are phosphoric acid salts of spermin ( $C_2H_5N$ ), or, as Majert and Schmidt give it,  $C_4H_{10}N_2$ , with the structure  $HN < \begin{matrix} CH_2-CH_2 \\ | \\ CH_2-CH_2 \end{matrix} > NH$ , thus being similar to, although not identical with, piperazin. The entire question of the composition of spermin is still unsettled, however; and it is probable, furthermore, that the crystals found in leukemia are not identical with the crystals observed in semen.

Crystals that appear similar are also found in empyema, and ascitic fluid, bone marrow, and tumors, and it has been suggested that they are derived from or related to the oxyphile granules of the eosinophiles.<sup>32</sup> This view implies an agreement with Gumprecht's opinion that the crystals seen in bone-marrow, sputum, etc., are not spermin, but of protein nature. As can be seen, the nature and significance of Charcot's crystals are, at the present time, quite undetermined.

*Corpora amylacea* have been found in sputum. These probably have no chemical relation to the amyloid of tissue amyloidosis but represent merely deposits of precipitated proteins in stagnating, protein-rich fluids, and probably have no constant chemical identity. The reports of excretion of true starch grains in tuberculosis sputum are to be discredited; undoubtedly they come from oral contamination or from inhaled starch dust.

<sup>31</sup> Deut. med. Woch., 1895 (21), 475.

<sup>32</sup> Literature, see Floderer, Wien klin. Woch., 1903 (16), 276; Predtetschensky, Zeit. klin. Med., 1906 (59), 29; Jacobsthal, Virchow's Arch., 1921 (234), 12.

## THE LOSS OF NUTRITION IN SPUTUM

Not a little consideration has been given to the question of the sputum as a loss of nutrition from the economy of the consumptive. This loss is chiefly in the form of protein, and von Hoesslin compiles the following table of the observations in the literature, giving the daily loss of nitrogen:

| DISEASE                      | AUTHOR          | MINIMUM | MAXIMUM | AVERAGE |
|------------------------------|-----------------|---------|---------|---------|
| Bronchitis.....              | Renk            |         |         | 0.23    |
| Bronchitis.....              | Lanz            | 0.187   | 0.810   | 0.395   |
| Tuberculosis.....            | Renk            |         |         | 0.75    |
| Tuberculosis.....            | Plesch          |         |         | 2.133   |
| Tuberculosis.....            | Magnus-Alsleben | 0.54    | 1.206   | 0.87    |
| Tuberculosis.....            | Lanz            | 0.205   | 1.701   | 0.660   |
| Gangrene.....                | Lanz            | 0.224   | 1.668   | 0.813   |
| Gangrene.....                | Orszag          | 0.067   | 3.248   |         |
| Bronchiectasis.....          | Falk            |         |         | 0.66    |
| Pneumonia.....               | Lanz            | 2.6     | 4.87    |         |
| Pneumonia before crisis..... |                 |         |         | 0.731   |
| Pneumonia after crisis.....  |                 |         |         | 0.384   |

The variations in different cases may be illustrated by the following table of Lanz's results:

| CASE NUMBER         | SPUTUM<br>AMOUNT<br>PER DAY | N IN<br>PER CENT OF<br>MOIST<br>SUBSTANCE | PROTEIN<br>IN PER CENT<br>OF MOIST<br>SUBSTANCE | TOTAL LOSS |         |
|---------------------|-----------------------------|---|---|------------|---------|
|                     |                             |   |   | N          | Protein |
| 1                   | 100                         | 0.2698                                    | 1.6862  | 0.2698     | 1.6862  |
| 2                   | 46                          | 0.7461                                    | 4.6631  | 0.342      | 2.1450  |
| 3                   | 120                         | 0.4023                                    | 2.5147  | 0.4827     | 3.0176  |
| 4                   | 40                          | 0.5153                                    | 3.1906  | 0.2053     | 1.2832  |
| 5                   | 120                         | 0.5656                                    | 3.5349  | 0.6787     | 4.2419  |
| 6                   | 60                          | 0.6884                                    | 4.3025  | 0.4130     | 2.5315  |
| 7                   | 120                         | 0.9331                                    | 5.8321  | 1.1198     | 6.9985  |
| 8                   | 38                          | 1.1459                                    | 7.1619  | 0.4354     | 2.7215  |
| 9                   | 36                          | 1.060                                     | 6.2375  | 0.3593     | 2.2455  |
| 10*                 | 75                          | 0.9318                                    | 5.8237  | 0.6981     | 4.3672  |
| 10                  | 175                         | 0.7102                                    | 4.4387  | 1.2428     | 7.7675  |
| 10                  | 90                          | 0.6903                                    | 4.3144  | 0.6213     | 3.8830  |
| 10                  | 245                         | 0.6943                                    | 4.3394  | 1.7010     | 10.6313 |
| 10                  | 120                         | 0.5706                                    | 3.5662  | 0.6847     | 4.2794  |
| Average of 10 ..... | 121                         | 0.72                                      | 4.49  | 0.87       | 5.34    |

\* Results on 5 consecutive days immediately before death.

Plesch,<sup>33</sup> who obtained higher figures than most other authors, calculated that an average of 4.8 per cent of the total absorbed calories were lost in the sputum in his cases, this constituting 38.54 per cent of the excreted calories lost by the body. Renk remarks that a consumptive, excreting daily even 0.75 gram of nitrogen in the sputum, is thus losing 6 per cent of the nitrogen requirement in fasting, and 3.8 per cent of the nitrogen requirement of a well nourished person. This loss is especially important since it consists so largely of the valuable nucleoproteins, as shown by the P<sub>2</sub>O<sub>5</sub> loss found by Plesch, as recorded in the following table, the figures being obtained with composite samples of the food and excretions for three days immediately before death in a case of actively progressing pulmonary tuberculosis.

| IN 250 CC. SPUTUM DAILY AVERAGE     | DAILY AMOUNT | IN PER CENT OF DRY WEIGHT | IN PER CENT OF MOIST WEIGHT | CALORIC VALUE |
|-------------------------------------|--------------|---------------------------|-----------------------------|---------------|
| grams                               |              |                           |                             |               |
| Dry substance.....                  | 22.0         |                           | 8.8                         | 102.82        |
| Nitrogen.....                       | 2.13         | 9.64                      | 0.85                        | 54.67         |
| P <sub>2</sub> O <sub>5</sub> ..... | 0.50         | 1.29                      | 0.20                        |               |
| Fat.....                            | 3.23         | 15.16                     | 1.39                        | 28.78         |
| Calculated N-free extractives ..... | 4.43         | 20.13                     | 1.77                        | 19.37         |

The total balance is as follows:

|                                     | INTAKE  | EXCRETED |        |        |        | BALANCE  |
|-------------------------------------|---------|----------|--------|--------|--------|----------|
|                                     |         | Urine    | Feces  | Sputum | Total  |          |
| N.....                              | 11.32   | 9.91     | 4.12   | 2.13   | 16.16  | -5.84    |
| P <sub>2</sub> O <sub>5</sub> ..... | 2.93    | 2.05     | 1.32   | 0.53   | 3.90   | -0.97    |
| Fat.....                            | 106.02  |          | 18.47  | 3.24   | 21.71  | +84.31   |
| Calories.....                       | 2520.85 | 163.54   | 348.09 | 102.82 | 614.45 | +1906.45 |

With the sputum was lost, therefore, 18.84 per cent of the nitrogen of the food, 3.06 per cent of the fat, 4.08 per cent of the calories. v. Hoesslin comments that the sputum cannot be left out of consideration in calculating the metabolic balance, especially as there are cases with greater loss than those cited above.

<sup>33</sup> Zeit. exp. Path., 1906 (3), 446 (literature).

As shown in the table on p. 255 there is sometimes also a slight loss of nutrition in fats, for 3 grams excreted daily, a figure that has been observed in tuberculosis, amounts to 27 calories. Probably the amount of carbohydrate lost is not of importance.

Inorganic salts lost in the sputum have been looked upon as entitled to consideration in the light of theories of demineralization. Ott<sup>34</sup> has studied the calcium and magnesium balance in a consumptive for four days, and gives the following figures in grams:

|                      | N     | CaO   | MgO   |
|----------------------|-------|-------|-------|
| Intake.....          | 71.82 | 18.29 | 1.67  |
| Excretion            | 68.93 | 1.10  | 0.32  |
| Urine.....           | 4.71  | 15.10 | 1.06  |
| Feces.....           | 1.54  | 0.064 | 0.008 |
| Sputum.....          | 75.18 | 16.26 | 1.39  |
| Excretion—Total..... |       |       |       |

These results do not indicate that the loss in these two elements in the sputum was of any significance in this case, at least. However, Falk<sup>7</sup> has estimated that the amount of certain salts lost in the sputum in some cases of tuberculosis may equal or exceed that in the urine, citing the following figures: In a patient who excreted 4.31 grams of inorganic salts per day in the sputum, the Cl loss in the sputum was greater than in the urine, the P<sub>2</sub>O<sub>5</sub> was the same in each. In a case of lung gangrene Salkowski found in a three-day period the average figures:

In urine, 1.29 grams potassium and 2.96 grams sodium  
In sputum, 1.26 grams potassium and 2.20 grams sodium

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<sup>34</sup> Deut. Arch. klin. Med., 1901 (70), 582.

## CHAPTER XI

### METABOLISM IN TUBERCULOSIS

Although there is a large literature on this subject, most of the older data are valueless because of inadequate methods, and even the best of modern work suffers because tuberculosis is not a simple disease. Not only does it present many stages and types of development, but it is usually accompanied by any and all sorts of complications, which must modify the metabolism. Acute secondary infections, pleural effusions, intestinal ulceration, hemorrhage, anemia, renal injury, amyloidosis, cardiac insufficiency and gastric derangements all have a decided influence, and in addition the analytical figures are complicated by the losses through the sputum and the drenching perspiration. Despite all these obstacles to accuracy we have some useful information on this subject, which is of the greatest importance, since it must serve as the basis for the suitable dietary management of the tuberculous patient.

Two main problems are presented. (1) Is the rate of energy transformation altered in tuberculosis? (2) Is protein metabolism altered either with the total metabolism or independently thereof?

### CALORIMETRY IN TUBERCULOSIS

The literature of this subject has been reviewed by McCann and Barr,<sup>1</sup> who discard most of the early work as largely without value. They recall, however, that in 1811 Nysten studied the oxygen and CO<sub>2</sub> of the expired air, even before the calculation of the energy transformation from the gaseous exchange was a possibility. The first valuable contribution they attribute to Hannover (1845), who studied the production of carbon dioxide in 5 patients with phthisis. By assuming an average respiratory quotient of 0.80, we may calculate the daily heat production of these patients as

<sup>1</sup> Arch. Int. Med., 1920 (26), 663.

between 1200 and 2100 calories per day, and from 26.5 to 40 calories per kilogram of body weight. In 1878 Moeller<sup>2</sup> studied poorly nourished patients with phthisis, males weighing from 44 to 45 kgm., aged from twenty-four to forty-eight years. The carbon dioxide produced was measured in the Pettenkoffer-Voit apparatus in six-hour periods. These patients all had food and beer, so that the metabolism was not basal. Assuming an average respiratory quotient of 0.80, the daily heat production would be between 1600 and 1780 calories, or from 35 to 40 calories per kilogram of body weight.

Loewy,<sup>3</sup> who studied exchange of oxygen and carbon dioxide, minute volume respired and nitrogen excretion, at different body temperatures after tuberculin injections, using the Zuntz-Geppert apparatus, found that there was only a moderate increase in oxygen consumption in fever and that it was greater with rising than with falling temperatures. Kraus and Chvostek<sup>4</sup> studied 5 phthisical patients with the same apparatus. The conditions were not basal, as the patients were not fasting. However, calculations show that an increase of 39 per cent in the metabolism increased the minute volume respired from 7743 cc. to 10,000 cc. in a case in which proper quotients were obtained.

Robin and Binet<sup>5</sup> give mass statistics regarding the minute volume, lung capacity, oxygen consumption and respiratory quotients of 162 phthisical patients. Averages only are given, the methods used are not stated, and there has been no attempt to compare individual patients with normals of the same size, so that the data are not very satisfactory. However, it is of interest to note that the minute volumes respired were from 80 to 110 per cent greater than those of the normals studied. They also found a consumption of oxygen from 70 to 100 per cent greater. The respiratory quotients were lower in the tuberculous patients. Lung capacity was also greatly reduced. On the other hand, Tissot and Charrin<sup>6</sup> found in tuberculous guinea pigs no increase in metabolism in the

<sup>2</sup> Zeit. f. Biol., 1878 (14), 542.

<sup>3</sup> Virchow's Arch., 1891 (126), 218.

<sup>4</sup> Wien. klin. Woch., 1891 (4), 104, 127.

<sup>5</sup> Bull. méd. Par., 1901 (15), 249.

<sup>6</sup> Jour. physiol. et path. gén., 1905 (7), 1009.

early stages of tuberculosis, and a decrease when the animals have begun to emaciate.

Staehlin<sup>7</sup> studied a tuberculous patient during a night sweat in a Jacquet respiration chamber. Food was taken before the observation and the subject was very restless. It was concluded, however, that the metabolism was not raised and that sweating had no influence on the total metabolism.

In a study of the effect of acetyl salicylic acid on the heat regulation in fever, Barbour<sup>8</sup> used three tuberculous subjects. These subjects were studied in a Benedict respiration chamber under basal conditions. The basal metabolism in these 3 cases was only from 3 to 4 per cent above the average normal.

Using the Atwater-Rosa respiration calorimeter of the Russell Sage Institute of Pathology, the metabolism of 15 cases of tuberculosis was most accurately studied by McCann and Barr.<sup>1</sup> The tuberculous lesions were not in all cases pulmonary, and the pulmonary cases were in various stages of advancement. Nevertheless it was found that the respiratory quotients were all within normal limits, indicating that there is no noteworthy qualitative change in the proportion of protein and non-protein combustion in tuberculosis. The calorimetric data for their 15 cases and the 3 described by Barbour, are summarized in the table on pages 266-267.

It will be noticed that in only 10 of the 15 cases were the observations basal, that is, complete rest in the fasting state. In these 10 cases the variation from average normal was from minus 3 to plus 15 per cent and Barbour's 3 cases lie within the same range; therefore it is evident that *afebrile* tuberculous lesions have very little effect upon the basal metabolism. In the table is also stated what the normal metabolism should have been for the same person when he was in health, before the emaciation of the disease had reduced the surface area, and it is found that the basal heat production is usually equal to or less than the estimated calories per hour for the same patient in health; hence the increased basal metabolism as calculated depends chiefly upon the reduction in surface area.

<sup>7</sup> Zeit. klin. Med., 1908 (66), 241.

<sup>8</sup> Arch. Int. Med., 1919 (24), 624.

| NUMBER | DIAGNOSIS   | CALORIES PER TWENTY-FOUR HOURS |                                |  |  |                                  |                                      |                           | REMARKS                       |
|--------|---|--------------------------------|--------------------------------|--|--|----------------------------------|--------------------------------------|---------------------------|-------------------------------|
|        |   | WEIGHT IN HEALTH<br>kgm.       | SURFACE IN HEALTH<br>sq. meter | ESTIMATED CALORIES<br>PER HOUR IN HEALTH | PRESENT CALORIES PER<br>HOUR OBSERVED<br>sq. meter | PRESENT SURFACE AREA<br>per cent | METABOLISM<br>FROM AVERAGE<br>NORMAL |                           |                               |
| 1      | Tuberculosis,<br>pleura and peri-<br>toneum           |                                |                                | 69.5                                     | 1.56   | +13                              | 1,668                                | Basal                     |                               |
| 2      | Tuberculosis,<br>lungs and lymph<br>glands            |                                |                                | 84.4                                     | 1.57   | +35                              | 2,026                                | Fever, 40°C.,<br>cough    |                               |
| 3      | Pleurisy with effu-<br>sion                           |                                |                                | 78.6                                     | 1.57   | +29                              | 1,886                                | 40.1 to 39.4°C.,<br>quiet |                               |
| 4      | Acute miliary tu-<br>berculosis                       | 69.5                           | 1.84                           | 75.4                                     | 75.5   | 1.70                             | +11                                  | 1,812                     | Basal                         |
| 5      | Tuberculosis, both<br>lungs, cavitation               | 74.0                           | 1.85                           | 71.2                                     | 63.9   | 1.48                             | +12                                  | 1,534                     | Basal                         |
| 6      | Tuberculosis, both<br>lungs, cavitation               | 61.5                           | 1.77                           | 66.4                                     | 59.0   | 1.53                             | + 2                                  |                           | Basal, afebrile               |
| 7      | Tuberculosis, both<br>lungs                           | 56.0                           | 1.60                           | 68.8                                     | 73.5   | 1.40                             | +15                                  | 1,598                     | Fever, restless               |
| 8      | Tuberculosis, both<br>lungs                           | 61.3                           | 1.75                           | 67.4                                     | 64.9   | 1.74                             | - 3                                  | 1,558                     | Fever, restless               |
| 9      | Tuberculosis, both<br>lungs (17 days<br>before death) | 51.0                           | 1.55                           | 62.0                                     | 72.0   | 1.41                             | +28                                  | 1,728                     | Fever, cough-<br>ing          |
| 10     | Tuberculosis, both<br>lungs and larynx                | 59.0                           | 1.74                           | 68.7                                     | 81.5   | 1.62                             | +27                                  | 1,956                     | Fever, cough-<br>ing          |
| 11     | Tuberculosis, both<br>lungs                           | 64.5                           | 1.78                           | 70.3                                     | 69.9   | 1.53                             | +15                                  | 1,678                     | Basal, afebrile               |
| 12     | Tuberculosis,<br>apices and hip                       | 61.0                           | 1.67                           | 66.0                                     | 65.6   | 1.54                             | + 9                                  | 1,512                     | Basal, afebrile               |
|        |   |                                |                                | 63.0                                     | 1.53   | + 5                              | 1,658                                | Basal, 38°C.              |                               |
| 13     | Tuberculosis, both<br>lungs, mitral en-<br>docarditis | 76.0                           | 2.04                           | 80.6                                     | 84.6   | 1.88                             | +14                                  | 2,030                     | Coughing,<br>38.6°C.<br>Basal |
| 14     | Tuberculosis, both<br>lungs, with cavi-<br>tation     | 62.3                           | 1.69                           | 66.8                                     | 77.6   | 1.53                             | +28                                  | 1,852                     | Fever, restless               |

| NUMBER | DIAGNOSIS  |                                 |   |                                       |                                    |  |   |                                | REMARKS |
|--------|--|---------------------------------|---|---------------------------------------|------------------------------------|--|---|--------------------------------|---------|
|        |  | WEIGHT IN HEALTH<br><i>kgm.</i> | SURFACE IN HEALTH<br><i>sq.<br/>meter</i> | ESTIMATED CALORIES PER HOUR IN HEALTH | PRESENT CALORIES PER HOUR OBSERVED | PRESENT SURFACE AREA<br><i>sq.<br/>meter</i> | METABOLISM VARIATION FROM AVERAGE NORMAL<br><i>per cent</i> | CALORIES PER TWENTY-FOUR HOURS |         |
| 15     | Tuberculosis, both lungs, with cavitation, chronic nephritis | 82.0                            | 1.98                                      | 78.4                                  | 70.7                               | 1.74   | + 3   | 1,697                          | Basal   |
| 16     | Cold abscess   |                                 |   | 61.8                                  | 1.50                               | + 4  | 1,483   | H. G. Barbour's case           |         |
| 17     | Tuberculosis, kidney   |                                 |   | 62.4                                  | 1.54                               | + 3  | 1,598   | Same                           |         |
| 18     | Acute pulmonary tuberculosis                                 |                                 |   | 67.2                                  | 1.63                               | + 4  | 1,613   | Same                           |         |

In the remaining observations, which were not basal, it was found that the heat production may be considerably increased by coughing, restlessness and high fever. Analysis of the figures shows a general tendency for an increased body temperature to increase the heat production, which is, of course, usual no matter what the cause of the fever may be. However, the increase attributable to fever alone seems not to be large, for in 1 case a rise of 1°C. in two hours without a chill was accompanied by only 2 more calories heat production in the second hour. But with a rectal temperature of 40°C. (104°F.) the metabolism may be 30 per cent above the average normal. It may be recalled that there is often a marked similarity in the symptoms of early tuberculosis and early hyperthyroidism. On the other hand, sometimes the basal heat production in tuberculosis may be less than the normal for the same patient when in health; in other words, the loss of weight may be accompanied by a reduction in total metabolism which more than compensates for the tendency to increase caused by the disease.

This last-mentioned fact may account for the apparently opposite results obtained by Grafe.<sup>9</sup> Direct calorimetry in a Jacquet

<sup>9</sup> Münch. med. Woch., 1920 (67), 1081.

apparatus in 10 *severe* afebrile cases of pulmonary tuberculosis showed in 7 an increase of from 20 to 36 per cent in the metabolism, although 2 showed no increase and 1 a very slight increase. It is to be noted that the subjects of this investigation were for the most part extremely emaciated, which of course produces a low surface area, and hence the high metabolic rate calculated on this basis may not have been so high in proportion to the original normal metabolism of the same person. Grafe found about the same rate of metabolism in phthisis with moderate fever as in the advanced afebrile cases, but when the temperature rises above 39°C. the figures rise to great heights (50 to 75 per cent increase) such as are seen sometimes in the early days of acute infections.

Grafe agrees with McCann and Barr that the *respiratory quotient* is about normal, ranging from 0.74 to 0.90 according to the state of nutrition and food taken. The low N excretion in the urine with the raised metabolism in his cases is noteworthy, for it generally was less than 7 grams per day. This he attributes to the effort of the undernourished organism to spare its most valued body material. While the total metabolism is increased, the protein metabolism falls so that it seldom constitutes more than 10 per cent of the total source of energy. He estimates that for the advanced cases of severe progressive tuberculosis, even with complete rest in bed, whether they do or do not have fever, the maintenance of nutrition requires 50 to 55 calories per kilogram, or 1500 calories per square meter of surface. In cases with high fever the figures are 60 to 65 calories per kilo or 1700 per square meter. McCann and Barr, however, found that the specific dynamic rise in metabolism is produced by protein feeding in the same degree in tuberculous as in normal subjects,<sup>10</sup> and hence suggest that it may be well to limit the protein intake during periods of activity of the disease, in order to reduce the amount of work required of the lungs. In the less severe cases which they studied, furthermore, they found the caloric requirement much less than estimated by Grafe, 1000 to 1200 calories per square meter, and warn against unnecessary forced feeding in such cases. Kocher<sup>10</sup> found that a mixed meal of 1000 calories increased the ventilation of the lungs, measured as minute volume, 18 to 20 per cent.

<sup>10</sup> Corroborated by Kocher, Calif. State Jour. Med., 1921 (19), 450.

In a later paper McCann<sup>11</sup> discusses further the dietary requirements in pulmonary tuberculosis. As the basal heat production varies from 1350 to 2030 calories, the energy requirement at rest in bed might be estimated as follows:

1. Calculate the twenty-four-hour heat production for a normal individual of the same sex, age, height, and weight.
2. Having estimated the basal heat production, if the afternoon temperature reaches 104°F., and if the patient has fever for only half the day, an increment of 15 per cent is added. The usual allowance for the specific dynamic action of food, with ordinary mixed diets, is 10 per cent. When such calculations are applied, the daily energy requirement is found to range from 1760 to 2640 calories while at absolute rest in bed, depending on the size of the individual and intensity of the febrile condition.

As regards the effect upon the heat production in tuberculosis the results of partaking of food are quite comparable with those obtained in normal individuals. The greatest increase in heat production occurs when protein is taken, next, fat, and least of all, with carbohydrates. Rubner found 5.8 per cent of the energy of ingested glucose appeared as the energy of specific dynamic action. For fat he found 12.7 per cent, but Magnus-Levy found only 2.5, and Murlin and Lusk found 4.1 per cent. With protein and fat the effect upon the breathing volume is proportional to the effect upon the heat production. With carbohydrates the increase of breathing volume is large, and out of all proportion to that of metabolism.

In view of these facts it seems wise, therefore, to select such a diet as will make the least demand upon the respiratory mechanism.

Pulmonary forms of tuberculosis present a peculiar problem from the standpoint of the metabolism, which depends for its maintenance upon the respiratory functions of the lungs. Anything that increases the metabolism requires that the lungs furnish a greater oxygen supply to the blood for the tissues, receiving in turn from the blood a greater amount of carbon dioxide for elimination. This increased exchange of respiratory gases involves the breathing of greater volumes of air, which is accomplished either by

<sup>11</sup> Amer. Review Tuberc., 1922 (5), 870.

increasing the rate of respiration, or by increasing the depth, or both. One of the earliest manifestations of pulmonary diseases is the reduction of the vital capacity. As the vital capacity becomes smaller, the limit to the amount of tidal air becomes proportionally reduced, so that in advanced cases the demand for an increased ventilation of the lungs must be met entirely by increasing the rate of breathing rather than the depth.

In some cases McCann reports it possible to maintain nitrogen balance, and even to retain nitrogen, when from 37 to 44 grams (571 to 679 grains) of protein each day are ingested, of which about one-half is from animal sources. The attainment of nitrogen equilibrium with such a small amount of protein is dependent on the ingestion of large amounts of carbohydrate and fat, sufficient to make the total caloric value of the diet from 1.7 to 2.4 times the basal energy requirement. He believes that failure to establish nitrogen balance on such low protein diets is due to failure or inability of the subject to ingest sufficiently large quantities of carbohydrate and fat, rather than to an inherently large wear and tear quota in tuberculosis. One of the disadvantages of the enormous diets that have been recommended from time to time lies in the frequent inability of the patients to manage the alimentary task represented by such intakes.

The final advice of McCann, based on the above considerations, is that satisfactory nutrition may be attained by the use of moderate quantities of protein (60 to 90 grams per diem), with the use of fat up to the limits of digestive capacity, and sufficient carbohydrate to bring the total caloric value of the diet to from 2500 to 3000 calories. Such a diet will produce the least demand upon the function of the damaged lungs. From the standpoint of diminishing the specific dynamic effects of foods, there is an advantage in dividing the diet into more than three meals.

According to Bauer<sup>12</sup> the hyperactive metabolism of hyperthyroidism is rarely accompanied by tuberculosis, and equally the diminished metabolism rate that follows castration in either sex seems to be unassociated with tuberculosis. McBrayer<sup>13</sup> found that in about one-third of the cases of chronic pulmonary

<sup>12</sup> Med. Klin., 1921 (17), 1045.

<sup>13</sup> Jour. Amer. Med. Assoc., 1921 (77), 861.

tuberculosis the basal metabolic rate is increased over 10 per cent together with increased blood sugar; in about one-fifth of all such cases there may be an increased metabolic rate with normal blood sugar, or just the reverse. Rarely are both decreased, or one normal and one decreased. Since hyperthyroidism consistently shows both an increased basal metabolic rate and an increased blood sugar, it is evident that data on these features do not help in differentiating slight hyperthyroidism from early tuberculosis.

#### LUNG CAPACITY

Although the amount of respiration does not ordinarily modify metabolism, nevertheless the question of vital capacity is of interest in this connection. This is described by Lundsgaard and Van Slyke<sup>14</sup> as follows:

The amount of air a person is able to expire after a maximum inspiration is called "vital capacity." The vital capacity does not, however, indicate all the air within the lungs. A certain quantity remains even after a maximum expiration; we call this "residual air." The sum of the vital capacity and the residual air, i.e., the total volume of air held by the completely filled lungs, is called the "total capacity," or "total lung volume." If one stops breathing half way between a normal inspiration or a normal expiration, there will be in the lungs a certain quantity of air greater than the residual air and less than the total capacity. We call this amount of air the "middle capacity." The difference between the middle capacity and the total capacity (all that can be breathed in after a half expiration) is called the "reserve air." The difference between middle capacity and residual air (all that can be breathed out after a half expiration) is called the "complementary air." In accordance with the definition now in use, the vital capacity is equal to the sum of the reserve and complementary air. Under normal conditions the difference between the inspiration and expiration (the tidal air) is much less than the vital capacity, and can be estimated approximately at 500 cc. This means that a person uses only 250 cc. of his reserve air and 250 cc. of his complementary air in normal breathing. The rest of the vital capacity is to be considered as a reserve which can be used if necessary under abnormal conditions.

In tuberculosis the lung volume has been studied extensively from the time of Hutchinson, who invented the spirometer in 1846 and studied the vital capacity in 22 cases of early and 9 cases of

<sup>14</sup> Jour. Exper. Med., 1918 (27), 65.

advanced pulmonary tuberculosis, finding the vital capacity subnormal in all. In the former group he found a decrease of from 10 to 50 per cent and in the latter from 40 to 80 per cent. This has been confirmed by many observers since, but according to Garvin, Lundsgaard and Van Slyke<sup>15</sup> few attempts have previously been made to show which of the factors affecting the vital capacity are responsible for its decrease in tuberculosis. They determined the total capacity, middle capacity and residual air in 31 adult male patients suffering from tuberculosis of the lungs. In 9 patients with incipient tuberculosis, the total lung volume was found within normal limits, whereas the vital capacity was diminished as a result of an increased residual air. The increase in the residual air was due to less complete expiration, caused partly by diminished movement of the diaphragm, partly by diminished compression of the chest wall. The diminished movement of the diaphragm was, as a rule, most marked on the most affected side. Whether these decreased movements are due to a reflex or to stiffness of the lung tissue they could not determine. The middle capacity was found practically normal. In 22 cases of moderately advanced and advanced tuberculosis, the total lung volume was in most cases markedly decreased. The vital capacity was substantially decreased, principally as a result of the diminished total capacity. The residual air was, as a rule, normal, although in a few cases an increase in residual air also contributed to the decrease in the vital capacity. The middle capacity was normal in some patients and considerably diminished in others.

Dryer<sup>16</sup> has developed vital capacity constants and applied them to the study of pulmonary tuberculosis. From the results of his investigation on a large number of cases he concludes that in pulmonary tuberculosis there is a definite decrease in vital capacity as compared with what should be normal for that same individual, an improvement in the clinical condition being accompanied by an increased vital capacity while an advance of the disease results in a decrease in the vital capacity. On this basis, the vital capacity is a valuable indication of the extent of the disease and as a measure of the effect of treatment.

<sup>15</sup> Jour. Exper. Med., 1918 (27), 87.

<sup>16</sup> Lancet, June 5, 1920, p. 1212.

*Oxidative capacity in pulmonary tuberculosis*

There is no experimental evidence that the loss of pulmonary tissue ever causes reduced oxidative activity through reduced oxygen supply, although such a condition is often found to be assumed in the literature of tuberculosis. As a support to the hypothesis that there is a reduced oxidative activity in the body in phthisis is the frequent occurrence of fatty changes in the liver. However, we have no positive proof that this fatty metamorphosis of the liver does depend upon reduced oxidation, but assume this pathogenesis since we find a similar condition in severe anemia and after poisoning with substances that are supposed to interfere with oxidation.<sup>17</sup> Also we have the fact that in the clinical forms of acidosis in which lactic acid is found in the urine there is usually associated fatty degeneration of the liver, and there is little doubt that lactic acid excretion is a true indication of defective tissue oxidation. While it seems reasonable to assume that there must be more or less reduction in oxidative activity in a disease in which so much of the lung is involved, yet we lack a demonstration that such is the case. On the other hand we have the exact studies of Carpenter and Benedict<sup>18</sup> on a man whose total lung volume was reduced about one-half through collapse of the left lung, who showed no appreciable alteration in the oxidative metabolism. Similar results were obtained in experimental animals by Takenaka,<sup>19</sup> who removed one of the lungs in rabbits and dogs, and found the respiratory metabolism and the nitrogen balance normal, despite a noticeable decrease in the oxygen content of the blood shortly after the operation, occurring with an increase in the hemoglobin and erythrocytes. Lippert<sup>20</sup> found that when an open pneumothorax was produced in rabbits there was a rapid fall in the oxygen content of the arterial blood, even to one-half the original figure, the oxygen content of the venous blood falls somewhat less, while the CO<sub>2</sub> is increased in both.

<sup>17</sup> See Wells' Chemical Pathology, 4th Ed., p. 409.

<sup>18</sup> Amer. Jour. Physiol., 1909 (23), 412.

<sup>19</sup> Kekkaku Zassi, 1918 (1), No. 1. See also Heuer and Andrus, Bull. Johns Hopkins Hosp., 1922, (33), 130.

<sup>20</sup> Beitr. Klin. Tuberk., 1912 (24), 389.

## PROTEIN METABOLISM

Probably because the technic involved is much simpler, the state of nitrogen equilibrium has been much more investigated than the gaseous exchange. There is a vast amount of literature on nitrogen excretion in tuberculosis, with and without comparison with the nitrogen intake, and most of this falls within the time covered by the review in Ott's work, written by May, to which the reader is referred for details and bibliography. The chief questions investigated have been: (1) Does the usual loss in weight depend on defective nutrition, on intoxication, or upon loss through fever? (2) Is there a toxicogenic tissue destruction in tuberculosis, and, if so, to what degree does it vary in the different stages and manifestations of the disease? (3) Under what conditions, if any, does the tuberculous patient maintain nitrogen equilibrium, or retain nitrogen? (4) To what extent are protein digestion and absorption impaired? (5) To what extent is nitrogen lost in the sputum and sweat?

Of these problems, the question of toxicogenic tissue destruction has been particularly considered, and is fully reviewed by May. By toxicogenic protein destruction is meant the reduction in the amount of tissue protein, as measured by a preponderance of nitrogen excretion over nitrogen intake, that takes place under the influence of tissue poisons and despite adequate alimentary supply of protein food. This must, of course, be distinguished from the deficient nitrogen balance that occurs in disease when insufficient absorption or food supply reduces the intake of N below the basal needs of the body. It is to be considered, in this connection, that fever itself, like work, is chiefly at the expense of the non-protein elements, provided these are furnished in sufficient amounts to protect the proteins. Thus, F. Voit found that artificially raising the temperature of a fasting dog to 40 to 41° for a period of twelve hours increased the N elimination 37 per cent above the normal, but if the animal were given 30 to 40 grams of cane sugar no increased protein metabolism occurred. Similar observations have been made in man. We may, therefore, exclude the fever *per se* as the cause of weight loss in consumptives, and if a nitrogen loss occurs despite adequate supply of non-protein

nutrition we are justified in assuming that a toxic action on the tissues is responsible. Conceivably this toxic destruction of tissues may be brought about by stimulating tissue breakdown, or by interfering with tissue repair.

### *Digestion and absorption of food*

As to the utilization of food, May found that there is no evidence of any serious interference with protein or carbohydrate absorption in tuberculosis, although in cases of local intestinal disease, fat absorption may be decreased.

Thus, Beiderert has described fat diarrhea in a case of caseation of the mesenteric glands through obstruction to the absorptive lymphatics. Another case in which there was much loss of fat, described by Müller, was accompanied by ulceration and amyloidosis of the intestines. Here there was a loss of 12.41 per cent nitrogen and 32.9 per cent fat, whereas the normal average loss through the feces is 10.6 per cent of nitrogen and 10.5 per cent of fat. Zahn reports a case with 15.7 per cent loss of fat in the feces in a consumptive without fever and with no intestinal ulcers or amyloid. By improved methods of gastric analysis, Mohler and Funk<sup>21</sup> have found that pulmonary tuberculosis causes a definite decrease in both the motility and the secretory function of the stomach from the very beginning of the disease; hyperacidity is rare. They consider that the swallowing of sputum, together with visceroptosis and gastrectasis, are responsible for this gastric deficiency. As far as fecal content of enzymes serves as an indicator of pancreatic function, this also is decreased in toxic states of tuberculosis according to the observations of Gray and Pickmann,<sup>22</sup> who found a constantly low fecal trypsin content a bad prognostic sign.

Of course in ulcerative enteritis there may be actual nitrogen loss from hemorrhage and exudation, but this is a form of body protein loss comparable to that occurring with the sputum, and does not properly enter into the consideration of metabolic

<sup>21</sup> Amer. Jour. Med. Sci., 1916 (152), 355.

<sup>22</sup> Jour. Amer. Med. Assoc., 1915 (65), 1271.

alterations. The importance of intestinal lesions in causing nitrogen loss has been well established by the observations of Wallace and Salomon.<sup>23</sup> They administered 250 grams of cane sugar daily to normal persons and to persons suffering from intestinal diarrhea and determined the amount of fecal-N during a period of two to three days, the results being given in the following table. It will be seen that in tuberculous enteritis the fecal-N loss equals half the urinary-N for fasting patients.

|  | N IN FECES<br>PER DAY |
|--|-----------------------|
|  | grams                 |
| Normal man.....                          | 0.539                 |
| Normal man.....                          | 0.380                 |
| Tuberculous ulceration of intestine..... | 3.075                 |
| Tuberculous ulceration of intestine..... | 4.186                 |
| Cancer of intestine.....                 | 1.74                  |
| Cancer of intestine.....                 | 1.974                 |
| Catarrh of intestine (severe).....       | 1.464                 |
| Catarrh of intestine (severe).....       | 1.087                 |

When there are no intestinal lesions the capacity of the alimentary tract to handle even excessively great quantities of food seems not to be impaired in pulmonary tuberculosis. This has been demonstrated by several clinical studies. We quote the following statement from a report by Austin, Ordway and Montagu.<sup>24</sup>

Among the earlier efforts in this direction was one by Goodbody, Bardswell and Chapman, in which, while the average daily food contained 3500 calories, in some instances it was carried to 4187 calories, and in one instance to 5026. Fat was unusually well utilized; in one individual, with an ingestion of 231.32 grams of fat during twenty-four hours, 96.41 per cent was absorbed. It was found that when a largely increased amount of fat was given a healthy individual, used as a control, the feces showed a large increase in this ingredient, but this was not true of the tuber-

<sup>23</sup> Med. Klin., 1909 (5), 579; quoted from Lusk.

<sup>24</sup> Boston Med. Surg. Jour., 1910 (163), 575.

culous. Vannini and Collina have also investigated the metabolism of 3 sufferers from pulmonary tuberculosis and found that when the gastro-intestinal tract was unaffected, the absorption did not differ much from that of the normal individual; there was, however, in 2 cases, a greater loss of nitrogenous material in the feces than is customary, but they were using a diet containing only 70 grams of albumin and a calory content of only 1800. The fat in the feces varied from 8.91 to 14.45 per cent of that contained in the food, a result much inferior to that found by the previous authors, who found a loss of only 3.59 per cent; the difference depends on the amount given, and we may conclude that with an increase in the amount of fat ingested there is apparently a less percentage of loss.

Any changes which may occur in the absorption of the tuberculous is due to the disease *per se* and not to the accompanying temperature, for Von Noorden's patient, suffering from tuberculosis, but with no temperature, when taking 11.6 grams of nitrogen daily in the form of albuminous food showed a loss of 8 per cent, and of the 85 grams of fat taken daily, a loss of 7 per cent. Six days later, with the same diet, but with a high temperature produced by the effect of tuberculin, the loss of nitrogen in the feces was 8.4 per cent and of fat, 7.3 per cent; this variation is so slight that it may be neglected. This principle is universally true, so that, as v. Hoesslin remarks, the presence of temperature in tuberculosis, if pulmonary, need not deter us from trusting the intestinal canal to elaborate and absorb these enormous quantities of food necessary to maintain the body weight.

Austin, Ordway and Montagu studied the utilization of food by three consumptives, the stage of the disease not being stated, who were receiving from 3989 to 6619 calories per day during five-day periods, with the proportion of proteins and fats varied. The following table (quantities in grams per day) shows that the absorptive power of the digestive tract is not at all impaired in these 3 cases of phthisis.

| PERIOD   | NITROGEN<br>INTAKE | NITROGEN<br>IN FECES | NITROGEN<br>LOST<br>IN FECES | FAT<br>INTAKE | FAT<br>IN FECES | FAT LOST<br>IN FECES |
|----------|--------------------|----------------------|------------------------------|---------------|-----------------|----------------------|
| Case I   |                    |                      |                              |               |                 |                      |
| 1        | 33.22              | 0.3925               | 1.18                         | 162.02        | 1.8308          | 1.13                 |
| 2        | 31.865             | 0.3606               | 1.13                         | 192.83        | 2.002           | 1.03                 |
| 3        | 31.64              | 0.396                | 1.25                         | 354.88        | 1.664           | 0.47                 |
| Case II  |                    |                      |                              |               |                 |                      |
| 1        | 33.081             | 0.4933               | 1.49                         | 160.54        | 5.082           | 3.65                 |
| 2        | 35.504             | 0.5469               | 1.53                         | 214.65        | 3.9003          | 1.81                 |
| 3        | 29.915             | 1.2612               | 4.21                         | 425.88        | 5.1565          | 1.21                 |
| Case III |                    |                      |                              |               |                 |                      |
| 1        | 29.715             | 0.6473               | 2.17                         | 158.71        | 4.9217          | 3.1                  |
| 2        | 28.963             | 1.0316               | 3.56                         | 212.375       | 6.4562          | 3.04                 |
| 3        | 25.523             | 0.9108               | 3.56                         | 371.2         | 4.8149          | 1.29                 |

#### *Loss of nitrogen in sweat*

The analytical difficulties in the way of accurate determination of loss through perspiration prevent more than approximate results either in tuberculosis or in other conditions, although it is to be assumed that the excessive perspiration of the consumptive may be sufficient to introduce errors in calculating nitrogen balance in metabolism studies. As far as known the qualitative composition of the sweat is not very different from that of the urine, and the nitrogen lost in the sweat cannot be considered as a loss of nourishment as is the case with the loss in the sputum, since it represents solely the nitrogenous end products of metabolism. We have some figures on the nitrogen content of the sweat. According to Argutinsky<sup>25</sup> the nitrogen content of sweat produced by a steam bath amounted to from 0.07 to 0.11 per cent, of which about 70 per cent was urea. Winternitz obtained in the sweat produced by a hot bath (maximum temperature 40.6°C., of three-quarter hour duration in a healthy man), a nitrogen loss of 0.6 gram, the quantity of sweat being estimated at about one liter. A man exposed to a light bath for fifty minutes, secreted 380 grams of

<sup>25</sup> Quoted by Winternitz in Brauer, Schröder and Blumenfeld's Handbuch der Tuberkulose, Bd. II, p. 77.

sweat containing 0.302 gram nitrogen. Probably the chief way in which perspiration itself modifies metabolism is by increasing the heat loss which requires a corresponding amount of heat production for compensation. There is no more loss of CO<sub>2</sub> from a moist than from a dry skin.<sup>26</sup>

### *Milk secretion in tuberculosis*

The influence of maternal tuberculosis on the chemical composition of milk seems to have received little attention. Ott<sup>27</sup> cites only analyses by Ludwig on the milk produced by 2 women with advanced tuberculosis, shortly after delivery. These showed a marked increase in the fat content, while the other components were about normal. This increase of the fat is not caused by the dehydration of fever, for it has been found that in other febrile diseases both the fat and the other solids decrease. A slight increase in fat has also been found in one case examined by Vernois and Bequerel. Possibly the high fat content is related to the lipaemia which has sometimes been observed in advanced tuberculosis, and which may be responsible for the fatty liver of tuberculosis. Ott, however, criticises these analyses because the milk was obtained shortly after delivery at which time wide physiological variations occur.

He states further that in mammary gland tuberculosis of cows, the milk from the diseased portion of the gland may at first be practically normal except for the presence of tubercle bacilli. Later the milk comes to resemble a yellowish serum containing fibrin-like floccules but no pus. It is then richer in protein and poorer in sugar and fat than the milk from the normal parts of the gland. There was also observed, at least in this case, a marked reduction in the calcium, magnesium and phosphate. Analyses of milk of cows with advanced pulmonary tuberculosis made by Blyth in 1879 showed no chemical changes of significance although he also found that the milk from tuberculous glands tended to approach serum in character with a high coagulable protein content and marked reduction in casein, fat and milk sugar.

A similar conclusion is reached by Monvoisin,<sup>28</sup> who gives the following analytical results:

<sup>26</sup> Plaut and Wilbrand, *Zeit. f. Biol.*, 1922 (74), 191.

<sup>27</sup> *Chem. Path. Tuberc.*, p. 154.

<sup>28</sup> *Recueil de méd. vet.* 1906 (83), 528; 1910 (87), 16; *Compt. Rend. Acad. Sci.*, 1909 (149), 644, 695.

*Milk in mammary gland tuberculosis*

| IN GRAMS PER LITER                  | NORMAL COW |        | TUBERCULOUS COWS WITHOUT MAMMARY LESIONS |         | TUBERCULOUS COWS WITH LESIONS OF QUADRANT OF MAMMARY GLAND FROM WHICH THE MILK WAS TAKEN |         | No. 7*  |
|-------------------------------------|------------|--------|--|---------|--|---------|---------|
|                                     | No. 1      | No. 2  | No. 3                                    | No. 4   | No. 5  | No. 6   |         |
| Acidity, as lactic acid.....        | 1.543      | 0.664  | 1.292                                    | 0.124   | 0.249  | 0.229   | 1.015   |
| Total nitrogen.....                 | 5.87       | 8.67   | 4.212                                    | 10.80   | 8.97   | 8.24    | 5.38    |
| Fats.....                           | 46.5       | 29.6   | 59.7                                     | 1.5     | 4.2  | 0.70    | 41.9    |
| Lactose.....                        | 43.5       | 29.8   | 43.9                                     | 0.0     | 0.0  | 0.0     | 30.0    |
| Dry solids.....                     | 142.3      | 126.05 | 147.5                                    | 129.30  | 97.6   | 73.4    | 139.15  |
| Inorganic matter.....               | 7.3        | 8.20   | 6.7                                      | 9.5     | 9.05   | 9.6     | 8.1     |
| Chlorine as NaCl.....               | 1.40       | 4.13   | 1.05                                     | 4.81    | 4.85   | 5.13    | 2.98    |
| Freezing point.....                 | -0.0550    |        |  | -0.0510 |  | -0.0510 | -0.0550 |
| Index of refraction at 15°.....     | 1.3434     | 1.3416 | 1.3442                                   | 1.3499  |  |         | 1.3437  |
| Elect. resistance at 18°, ohms..... | 240        |        |  | 116     |  | 153     | 207     |

\* The last two columns of figures are analyses of samples of milk from the same cow at a few days interval.

The cases of tuberculosis of the mammary gland were far advanced, and show that the diseased gland loses more and more its capacity to secrete milk and at last merely exudes a fluid closely resembling blood serum, as indicated by the following table:

| GRAMS PER LITER      | NORMAL MILK | TUBERCULOUS MILK | BLOOD SERUM |
|----------------------|-------------|------------------|-------------|
| Protein.....         | 38.5        | 72.4             | 75-80       |
| Fat.....             | 46.5        | 0.7              | 1-3         |
| Sugar.....           | 43.5        | 0.-2.0           | 1-2         |
| Ash.....             | 7.3         | 9.6              | 8.7         |
| Sodium chloride..... | 1.4         | 5.1              | 5.6         |

This change in the composition of the milk is not characteristic of tuberculosis, being also produced by other forms of mastitis, except that only in tuberculous mastitis is the acidity decreased, hyperacidity being seen in acute mastitis.

Strubell,<sup>29</sup> who gives an elaborate review of the question of the passage of substances from the maternal organism to the offspring, has obtained evidence that tuberculous antigens and antibodies are both transferable from the mother to the fetus by the placental circulation. They also appear in the milk in large amounts in actively immunized women and cows, the mammary glands playing an active rôle in this transfer. If tuberculous antibodies behave like antibodies in other diseases they should be particularly abundant in the colostrum.<sup>30</sup>

#### *Toxic protein destruction*

Many early authors, as Klemperer and Cornet, assumed the existence of a toxic protein disintegration in tuberculosis as in other febrile diseases, and there is no doubt that when fever occurs from toxic influences there is such a protein disintegration. To learn whether there is a toxic protein disintegration in afebrile tuberculous conditions, several investigations have been made, but May states that generally the cases selected were all rather advanced although at the time of investigation in no case was

<sup>29</sup> Beitr. Klin. Tuber., 1920 (45), 38.

<sup>30</sup> See Lewis and Wells, Jour. Amer. Med. Assoc., 1922 (78), 863.

there fever. These results he has compiled in the following table.<sup>31</sup>

| AUTHOR                 | PATIENT |        |                 | AVERAGE DAILY CALORIES SUPPLIED |          |                                  | AVERAGE DAILY N BALANCE<br>grams | REMARKS                                    |
|------------------------|---------|--------|-----------------|---------------------------------|----------|----------------------------------|----------------------------------|--|
|                        | Age     | Sex    | Weight<br>kilos | Total                           | Per kilo | Per cent of calories as proteins |                                  |  |
| Klemperer              | 27      | M.     | 48              | 2080                            | 43       | 20                               | +1.25                            |  |
| Klemperer              | 18      | M.     | 52              | 2000                            | 38       | 22                               | +0.59                            |  |
| Klemperer              | 40      | (?)    | 68              | 2360                            | 35       | 19                               | +3.97                            | Weight increase of 4 kilos in twelve weeks |
| Klemperer              | 31      | (?)    | 55              | 2090                            | 38       | 20                               | +3.23                            | Weight increase of 4 kilos in six weeks    |
| Albu                   | 11      | W.     | 29              | 1390                            | 48       | 20                               | +1.35                            | Advanced phthisis with cavities            |
| Blumenfeld             | 17      | W.     | 37              | 2000                            | 54       | 16                               | +1.80                            |  |
| Blumenfeld             | 42      | W.     | 56              | 2750                            | 49       | 18                               | +1.27                            |  |
| Ott                    | 25      | M.     | 47              | 1850                            | 40       | 24                               | -0.05                            |  |
| Mitulescu              | 27      | M. (?) | 1800            | (?)                             | 21       | -0.42                            |                                  |  |
| Mitulescu              | 49      | M. (?) | 1580            | (?)                             | 22       | -1.32                            | Defective absorption             |  |
| Mitulescu              | 19      | W.     | 67              | 1700                            | 25       | 23                               | +0.55                            |  |
| Mitulescu              | 57      | M. (?) | 1370            | (?)                             | 29       | +0.29                            |                                  |  |
| Mitulescu              | 36      | M. (?) | 1453            | (?)                             | 21       | -0.04                            |                                  |  |
| Mitulescu              | 14      | W.     | 52              | 1720                            | 33       | 20                               | +0.61                            |  |
| Mircoliu<br>(Soleri A) | (?)     | M.     | 77              | 3880                            | 50       | 20                               | +0.43                            | Without antiserum                          |
| Mircoliu<br>(Soleri B) |         |        | 78              | 3860                            | 50       | 20                               | +3.00                            | With antiserum                             |

A glance at the table shows the lowest balance to be -1.32 and the highest +3.97. The caloric need was in all cases covered. If we take into consideration, however, that most of the patients were already more or less emaciated and that they showed little or no retention in spite of abundant nutrition, it would seem that there must be even in these cases a toxic protein destruction, but the figures given are not proof of any very great disintegration

<sup>31</sup> Ott, Chem. Path. der Tuberkulose, p. 290.

under the existing conditions. These cases with N balance only signify that in spite of most abundant supply no retention follows, without showing that there actually has been destruction of organ protein.

May also discusses the recorded observations on the metabolism in febrile cases, which he divides into three types: (1) slight evening temperature; (2) remittent fever, morning 38.5° and evening up to 40°; (3) intermittent or hectic fever. The observations in the literature are collected in the following table:

| AUTHOR              | PATIENT |     |                 | AVERAGE DAILY CALORIES SUPPLIED |          |                                  | AVERAGE DAILY N BALANCE     |                                  | REMARKS  |
|---------------------|---------|-----|-----------------|---------------------------------|----------|----------------------------------|-----------------------------|----------------------------------|--|
|                     | Age     | Sex | Weight<br>kilos | Total                           | Per kilo | Per cent of calories as proteins | N LOST IN FEVER<br>per cent | AVERAGE DAILY N BALANCE<br>grams |  |
| A. Ott              | 26      | M.  | 61.7            | 2450                            | 40       | 26                               | 13.5                        | - 0.65                           | Type I, Slight fever only  |
| A. Ott              | 24      | M.  | 50.9            | 2200                            | 43       | 27                               | 6.1                         | - 0.49                           |  |
| A. Ott              | (?)     | M.  | 57.0            | 2590                            | 45       | 22                               | 10.2                        | - 0.27                           |  |
| Huppert and Rissell | 31      | M.  | 58              | (?)                             | (?)      | (?)                              | 22.0                        | - 12.0                           | Type II, Moderate fever, as in caseous pneumonia without mixed infection |
| P. Zahn             | 19      | M.  | 44              | (?)                             | (?)      | (?)                              | 12.0                        | - 5.45                           | Type III, Hectic, usually mixed infection                                |
| P. Zahn             | 26      | W.  | (?)             | (?)                             | (?)      | (?)                              | 28.0                        | - 9.53                           |  |
| Benedict            | 16      | M.  | 37              | 1081                            | 30       | 21                               | 8.1                         | - 3.87                           |  |
| A. Ott              | 27      | M.  | 59.5            | 2490                            | 41       | 18                               | 6.6                         | - 0.46                           | Type III, Hectic, usually mixed infection                                |
| A. Ott              | 25      | M.  | 51.2            | 2490                            | 49       | 18                               | 9.0                         | + 0.03                           |  |
| Mitulescu           | 42      | M.  | (?)             | 1600                            | (?)      | 24                               | 7.5                         | - 0.8                            |  |
| Mitulescu           | 31      | W.  | (?)             | 1450                            | (?)      | 21                               | 14.0                        | - 1.57                           |  |
| Mitulescu           | 29      | W.  | (?)             | 980                             | (?)      | 25                               | 18.0                        | - 1.51                           |  |

This tabulation shows that in contrast to the feverless cases, almost always the febrile cases show a negative nitrogen balance, although this is usually not large and it may be as great in mild as in severe cases; but this may depend upon pushing of the nourishment in the severe cases. Although these results indicate that rise of temperature is accompanied by an increased protein destruction,

the amount of increase of N loss does not correspond closely to the amount of increase in temperature. However, the loss of N in the sweat is not taken into account and in cases with much sweat this may be considerable. On the other hand, when there is much exudation into the lungs, as in the pneumonic type, some N retention may seem to occur. Taken altogether we find that in febrile cases of tuberculosis there is a true increased tissue protein destruction which becomes most pronounced in the acute caseous pneumonia.

Numerous observers have found that with severe dyspnea there may be a considerable loss in nitrogen (Araki),<sup>32</sup> and in late stages of tuberculosis this may account for a part of the negative N balance, but dyspnea was not present in the cases studied in the reports discussed above. Heliotherapy also stimulates protein metabolism.<sup>33</sup>

As a résumé of this subject we may put forward the thesis that according to the results of the metabolism observed in many consumptives, or to be exact in all consumptives at certain times depending on the production and absorption of toxic substances from the tuberculous lesions—a pathological increase of the protein disintegration occurs of the character of a toxic protein disintegration. [May.]

While in tuberculosis the deviation from normal is not great, it can become important in course of time.

That these changes in metabolism may be attributed to the tuberculosis itself, at least in cases where there is no evidence of other infections that might be held responsible, is shown by the fact that the reaction that follows injection of tuberculin may produce similar effects. Numerous observations have been made on metabolism in the fever thus produced, and the results are summed up by Mitulescu<sup>34</sup> as follows: (1) If the tuberculin injection does not produce fever the previously existing N balance is not altered. (2) With febrile reactions there is an increased N and P excretion, and, as during the fever the quantity of nourishment assimilated is lower, there must be an increased tissue destruction. (3) Repeated injections lead to an improvement of the utilization of protein. (4) Excretion of organic P increases during the reaction.

<sup>32</sup> Zeit. physiol. Chem., 1891 (15), 335.

<sup>33</sup> Koenigsfeld, Zeit. klin. Med., 1921 (91), 159.

<sup>34</sup> Deut. med. Woch., 1902 (28), 697.

(5) Urea and uric acid excretion are influenced by tuberculin only if there is fever, but with fever the uric acid excretion is increased constantly, sometimes 100 per cent. This is probably dependent on the fact that the leucocyte count in the blood increases progressively.

May<sup>31</sup> summarizes the results of the early studies in protein metabolism as follows: For the wasting away of consumptives the poisons absorbed from the tuberculous areas are responsible. The action of these is complex.

A. They act on the nervous system, especially on the hunger sense, sweat secretion and the heat center. In that they produce loss of appetite and even aversion to food, they lead to

B. Undernourishment. This itself again causes a decrease in the resistance of the organism and prepares the soil for spreading of the disease.

C. Large doses of the poisons cause, with individual variations, a direct injury of the cell protoplasm, for they have a strongly toxic action. The loss of protoplasm thus produced is not very great and is dangerous only when it continues for a long time and cannot be made up.

D. It seems that the regeneration of the protein loss from cells suffering under the influence of the poisons is hindered by these poisons and in severe cases even completely prevented, so that the regeneration cannot take place. We may refer to this as an injury to the regeneration or to the vitality.

E. Greater amounts of intoxication are generally accompanied with fever.

F. The metabolism of febrile tuberculous subjects follows the same laws as fever from other causes. There is an increased destruction of protein while an increase in fat destruction results only from dyspnoea or chills, in short from increased muscular activity.

The results on the protein metabolism in tuberculosis are similar to those obtained by more recent students of metabolism in febrile diseases. Thus, Kocher,<sup>35</sup> found that after giving a typhoid patient a diet containing carbohydrate in large amount with very little protein, it was not possible to reduce, during the febrile period,

<sup>35</sup> Deut. Arch. klin. Med., 1914 (115), 106.

the output of urinary N to the low level of the normal "wear and tear" quota of protein metabolism. Instead of excreting from 2.5 to 4 grams daily as a normal man would, the typhoid patient excreted 16 to 20 grams per day. Leyden and Klemperer found that with an intake of 10.6 to 11.7 grams of N, a pneumonia patient with high temperature excreted 21.9 to 24.7 grams of N. McCann and Barr,<sup>1</sup> in their work on metabolism in tuberculosis previously quoted, made observations on the N balance in 4 cases, and state that when the food nitrogen was reduced to the low level of from 3 to 3.5 grams per diem, with the addition of from 600 to 900 calories to the basal requirement the urinary nitrogen excretion fell to the level of 5 or 6 grams per diem, the patient remaining in negative nitrogen balance. This indicates a "wear and tear" quota greater than normal. The minimum nitrogen excretion noted by Kocher in a typhoid patient was somewhat higher (10.4 grams during fever, from 5.8 to 6.7 grams beginning convalescence) and was obtained at the expense of much more carbohydrate and fat. A toxic destruction of protein exists, therefore, in tuberculosis, but to less extent than in typhoid fever. They conclude that:

In view of the results in these 4 cases, it seems probable that many febrile tuberculous patients may be kept in nitrogen balance on diets containing from 60 to 70 grams of protein per diem. The amount of protein is much less than Voit's standard allowance (118 grams) for normal men, though somewhat greater than the normal minima of Chittenden and of Sherman (from 35 to 45 grams). It is much less than is fed to patients in many of the sanitaria for tuberculosis.

#### *Metabolism in scrofula*

A study of the metabolism of tuberculous and *scrofulous children* has been made by Ellen Ahlqvist.<sup>36</sup> She quotes a previous study by Lungwitz on 2 tuberculous and 1 scrofulous child, which showed an excessive nitrogen excretion. Her own study involved 22 children from four to nine years of age, including 3 normal controls. The results were, briefly, that the nitrogen intake was largest in the healthy children, averaging 3.9 grams of

<sup>36</sup> Studien über den Stoffwechsel tuberkulöser und skroföler Knaben. Helsingfors, Centralbuchdruckerei. Akademische Abhandlung 1920.

protein per kilo of body weight; next followed the tuberculous children with an intake of 3.6 grams and last the scrofulous with 3.2 grams per kilo. The absorption figures averaged 87.4 per cent in the healthy, 92 per cent in the tuberculous and 89.9 per cent in the scrofulous children. The healthy children averaged 60.8 per cent retention of the nitrogen intake, the tuberculous 13.4 per cent and the scrofulous 7.5 per cent. In relation to absorption the retention figures were respectively 21.4, 14.6 and 8.3 per cent showing the marked deficiency in nitrogen retention in tuberculosis. The fat intake was 2.5 grams, 3.3 grams and 2.5 grams per kilo, with absorption figures of 90.4, 95.2 and 93.6 per cent in the healthy, tuberculous and scrofulous respectively. The carbohydrate intake in the same order was 13.4, 10.3 and 11.0 grams per kilo. Calcium metabolism showed the same intake in both the healthy and the diseased children, magnesium somewhat higher in the latter, with a slight retention of calcium and a much greater retention of magnesium. These last figures give no evidence of the reputed demineralization of tuberculosis.

### *Purine metabolism<sup>37</sup>*

While the older literature contains numerous reports on this subject, they have little or no value because they are based on an erroneous understanding of the subject, and without consideration of exogenous sources of the urinary purines. Since we have had a clear understanding of purine metabolism this has been little investigated in tuberculosis. Presumably, as in other diseases, the amount of purine excretion in the urine will vary more or less directly with the amount of cellular destruction, and hence with the amount of leucocytic infiltration of the lung and of general leucocytosis, for the rate of disintegration of the tuberculous tissues themselves is so slow that this can scarcely be a factor in the urinary purine excretion. Von Jaksch<sup>38</sup> found in a case of febrile pulmonary tuberculosis that 11.03 per cent of the urinary N was in the purine fraction; in another case with amy-

<sup>37</sup> Discussion and literature given in Wells' Chemical Pathology, Chapter XXIII.

<sup>38</sup> Zeit. klin. Med., 1902 (47), 1.

loidosis it was 17.39 per cent. While these figures have little absolute value, since the diet was not considered, they have some significance since they were the highest purine figures obtained in his series of urinary analyses in various diseases.

We can find few other figures on purine excretion in tuberculosis since the time of Clemen's review in Ott, but it is to be assumed that it will fluctuate in direct proportion to the leucocytosis, modified somewhat by acute attacks in which tissue katabolism is increased with a rise in the purine excretion. Jonescu and Gruenberger<sup>39</sup> examined 2 patients with early pulmonary tuberculosis reacting to tuberculin while on a purine-free diet. Each showed a slight increase in purine excretion, involving more the basic than the uric purine-N. Each showed a leucocytosis (12,000, 14,000) which may account for the purine rise, although the authors think that there was probably some tissue destruction. The fact that the bases especially were increased suggests an interference with the normal steps of oxidation of purines to uric acid.

On the other hand, Mitulescu<sup>34</sup> reported a marked rise in uric acid excretion resulting from such tuberculin reactions as produce considerable fever.

Labbé and Vitry<sup>40</sup> found the average daily excretion of purine-N in advanced tuberculosis to be 0.68 grams per day, and 0.686 in a series of cases just before death. The total nitrogenous excretion being low, the ratio of purine-N to total-N was much above normal figures on a similar low purine diet (see p. 316).

Parkinson<sup>41</sup> analyzed the urine from 50 cases of pulmonary tuberculosis in different stages, obtaining on a diet nearly free from purines an increase of 57 per cent in the purine excretion in tuberculosis as compared with his controls on the same diet.

Pulay<sup>42</sup> has described in a case of apical pulmonary tuberculosis with lupus erythematodes a marked retention of uric acid, and advances the hypothesis that this sensitizes the skin to light and thus leads to the lupus.

<sup>39</sup> Zeit. klin. Med., 1909 (68), 295.

<sup>40</sup> Revue de Méd., 1912 (32), 818.

<sup>41</sup> Practitioner, 1906 (76), 219.

<sup>42</sup> Dermatol. Wochenschr., 1921 (73), 1217 and 1245.

## THE CREATININE COEFFICIENT

Since Folin discovered that the amount of creatine and creatinine in the urine is independent of the amount of protein in the food, and is constant in the individual, these substances have been regarded as indices of tissue katabolism. Normally the amount of creatinine excreted in the urine per day is in a fairly constant ratio to the degree of muscular development, and Schaffer<sup>43</sup> expresses this by the creatinine coefficient, which is the number of milligrams of creatinine-nitrogen eliminated per kilogram of body weight each twenty-four hours. In normal men this varies between 8 and 11, in women it is correspondingly lower.

It seems to be a general rule that fever of long duration produces a distinct increase of the creatinine and in many cases also of creatine, which may persist into the convalescent stage. Although this has generally been considered to be the result of the tissue destruction of a protracted fever, Bürger,<sup>44</sup> finding that the same things happened in febrile conditions of brief duration, believes that the hypercreatininuria can scarcely be considered as a manifestation of tissue loss, at least in all cases. He found that merely warming the muscles led to a hypercreatininuria with considerable amounts of creatine and suggests that the hyperemia of the muscles may be responsible.

Raphael and Eldridge<sup>45</sup> call attention to the fact that in tuberculosis there are operating two somewhat opposing factors, continued pyrexia and reduction in the muscle mass. They review the literature as follows:

But few cases of pulmonary tuberculosis have been reported in the literature from this point of view, and these show findings both indeterminate and in poor accord. Thus Hofmann,<sup>46</sup> employing a now obsolete procedure of creatinine determination and making no provision as to diet control, reports 1 advanced, though apparently afebrile case (patient aged thirty-four), in which there was noted a slight decrease of creatinine output —0.496 grams per twenty-four hours as opposed to his normal value of 0.687 grams. Van Hoogenhyze and Verploegh,<sup>47</sup> on the basis of Folin's

<sup>43</sup> Amer. Jour. Physiol., 1908 (23), 1.

<sup>44</sup> Zeit. Exper. Med., 1921 (12), 1.

<sup>45</sup> Arch. Int. Med., 1921, (27), 604.

<sup>46</sup> Virchow's Arch., 1869 (48), 358.

<sup>47</sup> Zeit. physiol. Chem., 1908 (57), 162.

technic, and utilizing a low protein diet, report a febrile case (patient aged seventy-two), marked by slight decrease in creatinine excretion—1.05 grams (average) per twenty-four hours. McClure,<sup>48</sup> also utilizing Folin's technic and employing a modified (purine and nuclein free) Shaffer-Coleman<sup>49</sup> diet, reports 4 febrile cases. The first case (patient aged nineteen), in which there was a positive Wassermann reaction, showed irregular increase with wide range (from 0.606 to 2.052 grams per twenty-four hours). In the second case (patient aged twenty-seven), complicated by hemopneumothorax, there was likewise noted occasional increase in creatinine output with a range of from 0.624 to 1.956 grams per twenty-four hours. In the third case, one of tuberculous broncho-pneumonia (patient aged twenty-eight), there was observed practically no increase and a range of from 0.607 to 1.291 grams per twenty-four hours, while, in the last case (patient aged twenty-five), also one of tuberculous broncho-pneumonia, there was found a slight decrease, with a range of from 0.540 to 1.000 grams per twenty-four hours.

Raphael and Eldridge<sup>45</sup> determined the creatinine output in three groups of 5 cases each of pulmonary tuberculosis, selected as representing three progressive grades of disease activity. Organically, these cases were all of "moderately advanced" or "far advanced" (third group) types, falling functionally or symptomatically, however, into the three groups of this classification, as follows: A, afebrile, absence of constitutional disturbance, ambulant and doing light work; B, slightly febrile, slight constitutional disturbance, in bed one-half day; C, definitely febrile, marked constitutional disturbance, confined to bed entire day and general condition serious to critical. The subjects were all males and care was taken to select no patients showing evidence of obesity and to exclude adolescent and senile types. They were placed on a strictly meat-free diet, and after a preliminary period of forty-eight hours, creatinine determinations were made on three successive days.

The findings were, for the most part, of consistent uniformity, with most variation in group A, and in all three groups the average values were definitely below the normal constant (8.1), as determined by Shaffer. On the other hand, there seems to be but little difference between the three grades, as compared with one another, save that the B cases appear, on the whole, to show a

<sup>48</sup> Arch. Int. Med., 1918 (22), 719.

<sup>49</sup> Arch. Int. Med., 1909 (4), 538.

slightly higher coefficient than either A or C. This might possibly be accounted for on the basis of the increased catabolism, due to fever and increased disease activity, not present in A, and not offset, as in C, by extreme wasting and general debility and asthenia. It would seem therefore, that in pulmonary tuberculosis, creatinine excretion increases with increase in disease activity, up to a certain point, at which a decrease becomes manifest, dependent, probably, on progressive tissue wasting and generally lowered plane of vital activity.

On the basis of the data secured in this study, it seems that, in clinically uncomplicated cases of pulmonary tuberculosis, the creatinine coefficient is slightly below the average normal figure,<sup>50</sup> and that it reaches its greatest height in middle grade cases, i.e., those in which increased catabolism, due to disease activity, has not yet been offset by excessive tissue waste and generally lowered vitality.

It is of interest to recall, in this connection, that McCann and Barr,<sup>11</sup> working with tuberculous patients from the point of view of calorimetric investigation, found that, due to weight-loss compensating for increase in metabolism dependent on disease activity, the basal heat production in this disease may represent a value definitely below that obtaining in health for the same individual.

#### MINERAL METABOLISM IN TUBERCULOSIS

Much attention has been given to this subject in the literature, because of the hypothesis, warmly supported by numerous French clinicians, that "demineralization" is a conspicuous feature of tuberculosis and responsible for the progress of the disease, with the resulting practice of "remineralization" therapy. Robin, Ferrier and others have written extensively on this subject, and as early as 1877 Senator stated that there is an excessive calcium excretion in tuberculosis. Sergent,<sup>51</sup> who reviews the early French work, was quite convinced of the value of calcium therapy. By

<sup>50</sup> Corroborated by Kocher, Calif. State Jour. Med., 1921 (19), 450.

<sup>51</sup> Presse Méd., 1910 (18), 865.

some the calcium loss was supposed to be the result of intestinal lesions (Loeper and Bechamp)<sup>52</sup> while others have suggested that it is the result of a binding of the calcium by toxic products of the tuberculosis.<sup>53</sup> Most of the early clinical chemistry on which rests the hypothesis of demineralization in tuberculosis, has been valueless, for the data have been obtained mostly by analysis of the urine without consideration of either the total intake of inorganic salts and their precursors, or the fecal excretion, which, in the case of calcium, is much greater than the urinary excretion. To base principles of diagnosis and treatment on such evidence is the height of absurdity.

Ott<sup>54</sup> seems to have been the first to study the mineral metabolism with knowledge of the intake of inorganic materials, and the output in both feces and urine. The total figures for each case studied are summed up in the table on page 293.

As far as can be deduced from this limited number of observations, a positive nitrogen balance is accompanied by a positive mineral balance, and a negative nitrogen balance by a negative mineral balance. (The loss of sulphur is due to failure to secure correct results on the food sulphur (Brasch)).<sup>55</sup> Some error, of course, creeps in from the failure to determine the loss in the sweat and sputum, but even so there is no evidence here of a demineralization as a characteristic feature of tuberculosis. Ott has also determined the balance of N, Ca and Mg in 5 other cases<sup>56</sup> of pulmonary tuberculosis in approximate nitrogen and caloric equilibrium and in all found no loss in either calcium or magnesium.

A. Mayer<sup>57</sup> determined the balances in 2 severe cases of phthisis in 12 years old children (Cases I and II), and three adults, one moderate (Case III) and 2 advanced cases shortly before death (IV and V), the results being given in the table on page 294.

<sup>52</sup> Compt. Rend. Soc. Biol., 1909 (67), 350; 1910 (69), 112.

<sup>53</sup> Croftan, New York Med. Jour., 1909 (89), 1182.

<sup>54</sup> Zeit. klin. Med., 1903 (50), 432.

<sup>55</sup> Deut. Arch. klin. Med., 1906 (87), 402.

<sup>56</sup> Deut. Arch. klin. Med., 1901 (70), 582.

<sup>57</sup> Deut. Arch. klin. Med., 1907 (90), 408.

Mineral metabolism in tuberculosis (*Ott*)

|   |                 | N      | K     | Na    | Ca    | Mg    | S     | P     | Cl    | TOTAL<br>ASH CAL-<br>CULATED |
|---|-----------------|--------|-------|-------|-------|-------|-------|-------|-------|------------------------------|
| Case I. Early apical pulmonary tuberculosis.                | Intake . . .    | 108.08 | 21.95 | 7.12  | 18.60 | 1.61  | 3.45  | 15.26 | 13.60 | 119.04                       |
|   | Excretion . . . | 97.95  | 19.97 | 5.55  | 17.50 | 1.34  | 6.14  | 15.13 | 10.71 | 116.59                       |
|   | Balance . . .   | +10.13 | +1.98 | +1.57 | +1.10 | +0.27 | -2.69 | +0.13 | +2.89 | +2.45                        |
| Case II. Moderately severe pulmonary tuberculosis four days | Intake . . .    | 84.37  | 16.35 | 5.43  | 14.45 | 1.24  | 2.74  | 11.55 | 10.58 | 92.31                        |
|   | Excretion . . . | 83.57  | 17.56 | 4.09  | 15.74 | 1.02  | 5.45  | 12.24 | 8.65  | 101.1                        |
|   | Balance . . .   | +0.8   | -1.21 | +1.34 | -1.29 | +0.22 | -2.71 | -0.69 | +1.93 | -8.70                        |
| Case III. Advanced pulmonary tuberculosis three days        | Intake . . .    | 61.01  | 14.39 | 3.89  | 12.14 | 1.00  | 1.83  | 10.08 | 7.36  | 74.53                        |
|   | Excretion . . . | 63.98  | 14.83 | 3.69  | 11.29 | 0.89  | 3.98  | 10.32 | 6.67  | 78.89                        |
|   | Balance . . .   | 2.97   | -0.44 | +0.20 | +0.85 | +0.11 | -2.15 | -0.24 | +0.69 | -4.36                        |

*Daily balances of inorganic elements in tuberculosis*

|          |                 | P <sub>2</sub> O <sub>5</sub> | CaO   | K <sub>2</sub> O | Na <sub>2</sub> O | Cl    | N     |
|----------|-----------------|-------------------------------|-------|------------------|-------------------|-------|-------|
|          |                 | grams                         | grams | grams            | grams             | grams | grams |
| Case I   | First day.....  | +1.32                         | +1.27 | -0.03            | +0.11             | +0.09 | -0.45 |
|          | Second day..... | +0.96                         | +0.84 | +0.04            | +0.15             | +0.04 | -1.20 |
|          | Third day.....  | +0.78                         | +1.09 | -0.13            | +0.01             | +0.27 | -1.38 |
| Case II  | First day.....  | +1.59                         | +0.49 | +0.13            | +0.04             | +0.38 | +1.95 |
|          | Second day..... | +1.53                         | +0.76 | +0.21            | +0.01             | +0.4  | +1.99 |
|          | Third day.....  | +1.54                         | +1.17 | +0.06            | +0.08             | +0.29 | +0.81 |
| Case III | First day.....  | +1.53                         | +0.35 | +0.60            | +0.25             | +0.45 | +0.53 |
|          | Second day..... | +1.33                         | +0.62 | +0.74            | +0.02             | +0.49 | +1.4  |
|          | Third day.....  | +1.57                         | +0.43 | +0.74            | +0.01             | +0.03 | +0.25 |
| Case IV  | First day.....  | +1.19                         | +0.29 | +0.14            | +0.02             | +0.75 | -1.1  |
|          | Second day..... | +0.77                         | +0.4  | -0.01            | +0.1              | +0.5  | -1.76 |
|          | Third day.....  | +1.5                          | +0.12 | -0.11            | +0.09             | +0.11 | -2.65 |
| Case V   | First day.....  | +1.98                         | +0.34 | -0.21            | +0.15             | +0.36 | -2.28 |
|          | Second day..... | +1.64                         | +0.13 | ±0               | +0.03             | +0.47 | -1.19 |
|          | Third day.....  | +1.38                         | +0.18 | +0.3             | +0.02             | +0.71 | -1.36 |

In all these cases the amount of phosphorus excreted in the urine was lower than the usual normal figures, which in adults on a mixed diet average 3.5 gram P<sub>2</sub>O<sub>5</sub> per day, and in twelve years old children, 2.25 grams. Hence there was a noteworthy phosphorus balance in spite of marked loss in nitrogen. It has been observed in other conditions that the body may retain phosphorus in considerable amounts despite a loss in nitrogen, but how to account for this retention is unknown.

Calcium, on the other hand, always exhibited a marked increase in the urine, but this was more or less compensated by a decrease in the feces, so that in all Mayer's cases there was a small plus balance of calcium. It will also be noted that there was a marked retention of chlorides with decreased urinary chlorides, depending chiefly on sodium chloride, since the potassium excretion was relatively increased although there was apparently a slight retention. It is to be considered that losses in sweat and sputum might readily amount to more than the apparent inorganic salts retention

in some of these cases. As Mayer points out, these results are merely what are commonly found in under-nutrition. Certainly they do not indicate that demineralization is an important feature of tuberculosis. The general conclusions of Mayer's work are supported by similar results obtained by Vannini.<sup>58</sup> While it has been argued that the tuberculous organism is salt-poor from the start, and hence negative salt balances are not observed in the metabolism studies, the tissue analyses of Steinitz and Weigert<sup>59</sup> show that there is no such deficiency in minerals in the tissues.

From Mayer's results it is seen that the urinary calcium excretion may be increased at the same time that the phosphorus excretion by this route is decreased. This is explained by von Noorden by the fact that the urinary calcium comes largely from the bones, while phosphorus may come from any tissue that is wasting.

#### *Calcium metabolism<sup>60</sup>*

Since calcified tubercles are usually sterile, healed tubercles, at least in man, numerous therapeutic attempts have been made to stimulate calcification of tuberculous lesions with the idea that thus healing would be accomplished. (See also Calcification of Tubercles, Chap. VI, and Calcium Therapy, Chap. XVI.) While we have no evidence that calcification favors the healing rather than follows healing, it is probable that a calcified tubercle is less likely to break down and liberate any remaining living tubercle bacilli than a tubercle that is not calcified. Whether administration of lime salts in any form or by any route will increase the rate of calcification is also problematical. Wersen<sup>61</sup> administered calcium lactate by mouth to 20 children with tuberculous peribronchial glands, and studied them by fluoroscopy along with an equal number of untreated controls. He believed that a much larger number of the treated cases showed calcification of the glands, together with a greater improvement in health. Of course the resistance to tuberculosis of those who work in lime dust is

<sup>58</sup> Bull. Scienz. Med. Bologna, 1908 (8), 341.

<sup>59</sup> Deut. med. Woch., 1904 (30), 838.

<sup>60</sup> See Eugen Schmitz, Dissertation, Düsseldorf, 1909; Wegelin, Corr. bl. Schweiz. Aerzte, 1910 (40), 913.

<sup>61</sup> Upsala Läkaer. Förh., 1914 (20), 1.

traditional,<sup>62</sup> but whether the calcium salts taken into the lung in these cases merely act mechanically to stimulate fibrosis, or are taken up and deposited in the tubercles, is not known.

While the experiments of Tanaka and others show that injected lime salts may temporarily increase the calcium content of the blood and lead to metastatic deposition of calcium salts, yet it has not been demonstrated that feeding of these salts will modify the blood calcium in tuberculosis. Denis and Minot<sup>63</sup> did not find any increase of calcium in the blood of normal persons given calcium lactate by mouth, 6 grams per day for six to ten days. Much calcium is normally excreted into the bowel, and hence it is quite probable that amounts appreciably raising the normal blood level will not be absorbed from the bowel. Nevertheless, as Givens<sup>64</sup> and others have found that feeding calcium salts causes an increased excretion of calcium in the urine it seems probable that some absorption takes place in the alimentary canal, although the calcium may be so rapidly removed that it does not cause any appreciable rise in the blood calcium.

Furthermore, some observers have found that when considerable amounts of calcium are fed there may be a retention of calcium (Voorhoeve,<sup>65</sup> Towles<sup>66</sup>). Thus, Mason<sup>67</sup> found that a 5 gram dose of CaCl<sub>2</sub> might cause a rise of plasma Ca by 2.5 mg. per 100 cc. in three hours; no effect was produced by calcium lactate. Increased calcium content of the tissues has been found in a few experimental animals receiving calcium (Kost).<sup>68</sup>

Howland and Marriott<sup>69</sup> found that when 0.5 to 1 gram of calcium chloride was given to children with tetany, which disease is accompanied by a fall in the serum calcium from the normal 10 to 11 mgm. per 100 cc. to from 4 to 7 mgm. (average 5.6 mgm.)

<sup>62</sup> See Reckzeh, Berl. klin. Woch., 1903 (40), 1022; Selkirk, Brit. Med. Jour., Nov. 14, 1908; Tweddell Med. Record, 1922 (101), 141. Fisac, Siglo Medico, Madrid, 1916 (63), 717.

<sup>63</sup> Jour. Biol. Chem., 1920 (41), 357.

<sup>64</sup> Jour. Biol. Chem., 1918 (34), 119.

<sup>65</sup> Biochem. Zeit., 1911 (32), 394.

<sup>66</sup> Amer. Jour. Med. Sci., 1910 (140), 100.

<sup>67</sup> Jour. Biol. Chem., 1921 (47), 3.

<sup>68</sup> A. Kost, Inaug. Dissert., Bonn, 1913.

<sup>69</sup> Quart. Jour. Med., 1918 (11), 289.

there occurs a prompt disappearance of the tetany together with a rise of the blood calcium, sometimes to normal figures, but sometimes, despite continued calcium feeding, the serum calcium could not be sent above 7.5 to 9 mgm. Jacobowitz,<sup>70</sup> however, was not able to increase the blood calcium by oral administration, either in normal children or in those with tetany, even when the therapeutic results are excellent. Denis and Minot also observed that sometimes in animals with a low blood calcium the figure rose somewhat when calcium salts were fed, but normal figures could not be raised. It is quite to be expected that figures lower than normal can be raised to normal, but that higher figures are not to be obtained, for we have it well shown by the phenomenon of metastatic calcification that the normal blood calcium represents about the maximum amount that the blood can carry, since greater amounts are promptly precipitated out in those tissues where the alkalinity is greatest.<sup>71</sup>

The experiments of Heubner and Rona<sup>72</sup> show that even subcutaneous injection of nearly lethal doses of  $\text{CaCl}_2$  (0.25 grams per kilogram) in cats produces a rise of only about 40 per cent in the blood calcium, reaching its maximum in thirty to ninety minutes and falling to normal within five or six hours. Intravenous injections of maximal quantities may drive the blood calcium up to two or three times the normal, but the original figure is restored in about two hours, thus indicating the capacity of the body to restore the blood calcium to normal under even extreme conditions.

Maver and Wells<sup>73</sup> have investigated the calcium content of the tissues of normal guinea pigs fed on the usual laboratory diet of hay, oats and carrots; of normal guinea pigs on the same diet with the daily addition of 0.1880 gram calcium lactate six days a week for several weeks; of guinea pigs infected with tuberculosis and given calcium lactate, and of guinea pigs similarly infected but not given calcium lactate.

The results may be summarized briefly as follows:

<sup>70</sup> Jahrb. f. Kinderheilk., 1920 (92), 25.

<sup>71</sup> For full review of subject of metastatic calcification see Wells, Arch. Int. Med., 1915 (15), 574.

<sup>72</sup> Biochem. Zeit., 1919 (93), 187.

<sup>73</sup> Amer. Review Tuberc., 1922 (6), 649.

1. Analysis of the tissues of *normal guinea pigs fed on the ordinary laboratory diet* without addition of calcium, shows that although there are marked individual variations in the calcium content of the same organ in different animals, and between the calcium content of different organs of the same animal, yet when the averages are obtained the proportion of calcium in each tissue is nearly the same, varying between 38 mgm. per 100 grams dry substance in the liver and 52.8 mgm. in the lymph glands.<sup>74</sup> If we estimate the average dry weight of the tissues as 25 per cent of the total moist weight, we find that the proportion of calcium in the tissues is from 10 to 14 mgm. per 100 grams moist weight, or quite the same proportion as in the whole blood of the animal.

2. Analysis of the tissues of *normal pigs fed calcium* shows, on the whole, little difference from the quantities found in the animals that had received only the usual food. The chief difference to be seen is an occasional exceptionally high result in the calcium fed pigs which brings up the average slightly above that seen in the control animals. It is not established by these analyses that as a general rule the normal animals that receive calcium have more calcium in their tissues than the control animals. There is no discernible difference in the calcium content of the animals that had received calcium for short or for long periods. Similar results were obtained with mice, in which analysis of the entire animal showed no more calcium in those given excess calcium than in those on normal diet.

3. Consideration of the results with *tuberculous animals* shows that while the organs which do not have tuberculous lesions (heart, kidneys, muscle), contain much the same quantity of calcium as the same organs of non-tuberculous animals, yet in the organs showing the most marked lesions (spleen and lymph glands) the proportion of calcium is greatly increased. This illustrates the tendency of calcium to deposit even in actively progressing tuberculous lesions,<sup>75</sup> but it is to be noted that the amount of

<sup>74</sup> Possibly the fact that the peribronchial glands were included in the analysis accounts for the fact that the lymph gland figures run high, since a certain amount of inhaled calcareous dust might be present.

<sup>75</sup> Caldwell (Jour. Infect. Dis., 1919 (24), 81) also found that tuberculous tissues which do not contain any macroscopic evidence of calcification often have a very high calcium content.

calcium in the tuberculous spleens and glands often happens to be much greater in the animals that *did not* get calcium. When local tuberculous lesions were produced in the testicle the diseased organ usually showed more calcium than the normal organ, alike in amount whether the animal had received calcium or not.

Hence these experiments do *not* indicate that administration of large amounts of calcium causes larger amounts to be deposited in tuberculous lesions than will be the case in an animal on a normal diet which contains ordinarily adequate amounts of calcium. In considering the value of calcium in human tuberculosis it is to be considered that tuberculous patients usually receive large quantities of milk, which contains about 1 to 2 grams of CaO per liter, thus insuring an adequate supply of calcium without any therapeutic administration of this element.

We know of no reliable figures by modern methods which show any deficiency in calcium in the blood in pulmonary tuberculosis. Halverson<sup>76</sup> and his colleagues have made careful analyses of the blood in patients with pulmonary tuberculosis, and draw the following conclusions:

Determination of the calcium content of the blood of patients with advancing and convalescing tuberculosis revealed that in the incipient cases in which the patients, who were on a high milk diet, showed marked improvement, the values for calcium in the serum were normal and fairly constant. In no case was the calcium value increased above normal figures by this high calcium diet.<sup>77</sup> In advanced cases the variations were greater (some rather high and some rather low values being obtained), and improving patients showed on the average slightly higher values than the unimproved. No marked deviations from the normal, however, were observed in the calcium content of the serum of patients in the various stages of pulmonary tuberculosis.

On the other hand some decrease in the plasma calcium<sup>78</sup> has been found to be a usual occurrence in pneumonia.

In view of the results recorded above we cannot lay much weight on the statement by Robin<sup>79</sup> that in 2 cases he found the

<sup>76</sup> Jour. Amer. Med. Assoc., 1917 (68), 1309.

<sup>77</sup> Corroborated by Muggia, Riforma med., 1921 (37), 1050.

<sup>78</sup> Davis and Talbot, Amer. Jour. Dis. Chil., 1921 (21), 29.

<sup>79</sup> Albert Robin, Treatment of Tuberculosis, trans. by Leon Blanc, Churchill, London, 1913, p. 27.

inorganic elements in the blood reduced to 6.38 and 7.02 grams per kilo (normal 8.392 to 9.109) and that one analysis showed a tuberculous lung to contain less inorganic matter than a normal lung, and the unaffected parts of the lung to contain less inorganic material in active tuberculosis than in chronic tuberculosis.

Labbé and Gallippe<sup>80</sup> studied the urine and fecal excretion of patients on a fixed diet, and found little difference between 5 normal and 20 tuberculous patients. They give the following figures:

Urinary excretion for twenty-four hours in 5 normal patients,  
 $\text{CaO}$ , 0.34;  $\text{MgO}$ , 0.19;  $\frac{\text{MgO}}{\text{CaO}}$ , 0.56.

Urinary excretion for twenty-four hours in 20 tuberculous patients,  $\text{CaO}$ , 0.30;  $\text{MgO}$ , 0.14; ratio 0.46.

Total average excretion in 7 cases of tuberculosis in different stages:

|                                | FECAL | URINE | TOTAL |
|--------------------------------|-------|-------|-------|
| $\text{CaO} \dots \dots \dots$ | 2.37  | 0.23  | 2.60  |
| $\text{MgO} \dots \dots \dots$ | 0.21  | 0.11  | 0.32  |

The total amount of bases eliminated in twenty-four hours was not found to be greater in tuberculous than in normal subjects; if anything it was less, thus agreeing with Mayer. It may be recalled that Ahlqvist<sup>36</sup> found retention of calcium and magnesium in tuberculous children.

Voorhoeve<sup>81</sup> points out, that the actual calcium balance in a tuberculous patient is of no significance, since decalcification is a relative term, depending on the amount of calcium supplied. He therefore studied carefully in a few cases the amount of calcium that was needed in the diet to maintain calcium balance, and found no very striking difference between his tuberculous and non-tuberculous subjects. Certainly if any tendency to decalcification was present at all it was but little marked, but the number of cases was not sufficient to permit of drawing any conclusions.

<sup>80</sup> Compt. Rend. Soc. Biol., 1913 (72), 876.

<sup>81</sup> Deut. Arch. klin. Med., 1913 (110), 231.

However, McCann<sup>11</sup> states that incomplete studies suggest that more calcium is required for maintenance of equilibrium in tuberculous than in normal subjects. The prevalent belief that consumptives suffer from dental caries because of abstraction of calcium could not be confirmed by Kiehnle.<sup>82</sup>

### *Magnesium metabolism*

*Magnesium metabolism* in tuberculosis has not usually been studied as distinct from calcium metabolism, nor has it been studied in other diseases for that matter as far as we can learn. Normally there is a much smaller proportion of the Mg excreted in the feces as compared with Ca, 30 to 40 per cent of it passing out in the urine, which contains usually 0.2 to 0.3 gram MgO per day. In starvation the proportion of Mg to Ca in the urine falls, presumably because of resorption of Ca from the bones. The only controlled metabolism studies that we can find on tuberculous patients are the 3 cases of Ott<sup>54</sup> each of which showed a slight Mg retention, despite some Ca loss in 1 case. Also the observation of retention of both calcium and magnesium in tuberculous children by Ahlqvist.<sup>36</sup> Dubard and Voisinet<sup>83</sup> report finding an excess excretion of magnesium in rapid, severe tuberculosis, and say that an excessive magnesium excretion in early tuberculosis means a lowered resistance. A corresponding phosphaturia is not always present.

### *Silica metabolism*

Some German investigators and clinicians have attributed much therapeutic value to silicates in tuberculosis, inspired largely by the pharmacologist, Kobert, who has emphasized the supposed importance of SiO<sub>2</sub> in the growth and strength of connective tissue.<sup>84</sup> The amount in the urine is said to be from 0.0614 to 0.2592 gram per day (Schulz), depending largely on the diet, most of the silica

<sup>82</sup> E. Kiehnle, Führt die Tuberkulose in ihrem Verlauf zu einer vermehrten Cariesfrequenz. Dissertation, Erlangen, 1921.

<sup>83</sup> Jour. des Practiciens, 1920 (34), 275.

<sup>84</sup> Early literature by Siegfried, Arch. de Pharmacodynamie, 1901 (9), 225; Hugo Schulz, Pflüger's Arch., 1901 (84), 67; 1902 (89), 112.

absorbed coming from vegetable foods. Kahle<sup>85</sup> reports that in 3 cases of phthisis the amount of SiO<sub>2</sub> in the urine was low (0.007 to 0.0093 gram per day) but he says nothing about the diet. In 3 other cases he found from 0.0076 to 0.015 gram but gives no normal figures that he has obtained himself.<sup>86</sup> He also found that the pancreas in active tuberculosis contained but 0.0828 gram SiO<sub>2</sub> per kilo dry weight, while under normal conditions the figures were 0.14 gram; in a case of healed pulmonary tuberculosis there was 0.2273 gram and in 2 chronic cases, 0.2047 and 0.1835 gram and in 1 case of very active post partum tuberculosis there was no silica in the pancreas. He assumes that the pancreas plays an active rôle in silica metabolism, but no comparative analyses of other tissues are presented to support this hypothesis. In his later paper he reports 20 analyses of the pancreas in pulmonary tuberculosis and says that his figures varied from 0.01 in active caseous pneumonia to 0.28 in healed tuberculosis. He also says that administration of absorbable silica compounds to tuberculous guinea pigs renders the lesions more fibrous, although without healing the process. These observations will require confirmation before they are generally accepted.

Further discussion of silica in tuberculosis will be found under Pneumonoconiosis (Chap. VI) and Silica Therapy (Chap. XVI).

#### *Phosphorus metabolism<sup>87</sup>*

The literature contains several statements concerning the excretion of inorganic phosphorus in tuberculosis, most of which are of little value because the intake of phosphorus is not considered, but only the urinary excretion, which is of little significance if we do not know whether we are dealing with endogenous or exogenous phosphorus. Thus, Zickgraf<sup>88</sup> states that Vierordt gives the normal daily "phosphate" (P<sub>2</sub>O<sub>5</sub>, presumably) excretion as averaging 3.0 to 3.5 grams, with variations from 1.6 to 5.58 grams. Zickgraf reports that he found variations only between 1.4 and

<sup>85</sup> Münch. med. Woch., 1914 (61), 752.

<sup>86</sup> Beitr. Klin. Tuberk., 1921 (47), 296.

<sup>87</sup> Full review given by Forbes and Keith, Phosphorus Compounds in Animal Metabolism, Tech. Bulletin, No. 5, Ohio Agric. Exp. Station, 1914.

<sup>88</sup> Zeit. inn. Med., 1910 (31), 273.

2.7 grams with an average of 2.25 grams but says nothing concerning the diet, nor does he in reporting that in 50 patients with early tuberculosis the average excretion was 2.36 grams.

In 1894 Tessier advanced the idea that an excessive excretion of phosphates, especially earthy phosphates, was a sign of value in the differentiation of tuberculosis from chlorosis in which the phosphate excretion was, he believed, lower. This could not be corroborated by Zickgraf, who found no essential difference in the urinary phosphates in the two diseases. Numerous other French observers have supported the view that demineralization, with loss of earthy phosphates in the urine as a prominent feature, is an important characteristic of tuberculosis, a view that has not been generally confirmed of recent years. Ott<sup>54</sup> is one of the few who has made suitably controlled investigations, and although he did find some evidence of demineralization this was not constant and early, the same being true for loss of phosphorus. Rechla Mulier<sup>89</sup> also made analyses in 10 cases of pulmonary tuberculosis of varying severity, and although the patients are recorded as receiving diets with  $P_2O_5$  content varying from 3 to 7 grams per day, the urinary  $P_2O_5$  showed no essential differences in respect to the amount or the percentage of the urine it constituted. If anything, the  $P_2O_5$  excretion observed in tuberculosis by both Zickgraf and Mulier was below the usual excretion of normal persons; which is in agreement with the findings of early investigators. Thus, Zapolsky in 1870 reported that in tuberculosis the total phosphate excretion is low, although the earthy phosphates are nearly normal, and Stokvis found low figures in pulmonary tuberculosis as compared with other lung diseases. Hirschfeld also found little or no increase to follow tuberculin reactions. The table of Mayer's results (p. 294) shows that he also found in tuberculous patients with analyzed diets, a decreased excretion of phosphates, apparently with some retention of phosphorus, despite the fact that both the urinary and fecal calcium excretion was increased. This agrees with the observation repeatedly made in studying metabolism, both under normal and fasting conditions, that the body retains its phosphorus with greater avidity than any

<sup>89</sup> Inaug. Dissert., Zürich, 1911, "Ueber Phosphorsäureausscheidung bei Lungentuberkulose."

other element, for when more than a minimum supply is given retention of phosphorus usually occurs.

In view of the above findings it is difficult to accept as a general rule the loss of phosphorus, which Mitulescu<sup>90</sup> found to occur sometimes when there was a positive N balance, although usually the greater the nitrogen loss the greater the loss in P. That any phosphorus loss that does occur comes from cellular disintegration is supported by the observations of von Jaksch and others that there may be a very high uric acid excretion in tuberculosis. However, we can find no studies of phosphorus and purine excretion in the same patients, which are necessary to give a full interpretation of the P losses.

*Organic phosphorus.* Normally a considerable fraction (2.5 to 4 per cent) of the urinary phosphorus is in organic combinations, supposedly glycero-phosphoric acid from lecithin and perhaps incompletely oxidized fragments of nucleic acid.<sup>91</sup> This seems to be entirely independent of the composition of the food, and hence is a measure of incomplete tissue metabolism.

We find that many years ago writers began to report a relative increase in organic phosphorus excretion in tuberculosis. Lépine and Eyommet<sup>92</sup> found the organic phosphorus in normal urine to be from 0.15 to 0.3 per cent of the amount of total nitrogen of the urine; in tuberculosis with fatty liver they found 1.0 to 1.8 per cent, but no increase in tuberculosis without fatty liver, so it was assumed that this organic phosphorus came from lecithin of the liver. They considered it to be chiefly or entirely glycerophosphoric acid.

In 10 cases of pulmonary tuberculosis Symmers found a marked increase in organic phosphorus, his figures being 0.20 to 0.99 gram per day, and in proportion to the total phosphorus from 9.4 to 80 per cent (averages 0.424 gram per day, and 26.6 per cent of the total phosphorus). This was without reference to hepatic changes, and it is to be noted that he found his highest figures not from lecithin but from nuclein destruction, in leukemia.

<sup>90</sup> Cent. inn. Med., 1902 (23), 1065; Zeit. f. Tuberk., 1903 (4), 513.

<sup>91</sup> See Symmers, Jour. Path. and Bact., 1904 (10), 159.

<sup>92</sup> Compt. Rend. Soc. Biol., 1882 (34), 622.

Mitulescu<sup>93</sup> found that in tuberculosis the proportion of organic phosphorus in the urine is higher than normal, which he believes indicates an increased destruction of tissues, with elimination of incompletely oxidized tissue phosphorus compounds.

### *Sulphur metabolism*

On a mixed diet of ordinary proportions, the normal sulphur excretion is about 0.7 to 1.0 gram per day, of which 0.10 to 0.25 gram is inorganic combination. Most of the sulphur taken with the food, whether organic or inorganic, is excreted oxidized as sulphates, and the urinary organic sulphur is believed to be chiefly of endogenous origin and therefore a measure of destructive tissue metabolism. Of the organic sulphur, part is in the form of sulphates of organic bases, such as indole and skatole, and hence this "ethereal" sulphur represents intestinal putrefaction. The "neutral sulphur" fraction includes the incompletely oxidized cystine and cysteine, and polypeptides containing these amino-acids, together with various other forms that are less understood, all presumably derived from protein decomposition. A considerable part of this neutral sulphur is found in the colloidal-nitrogen or proteic acid fraction of the urine (see p. 327), in large part in the chromogen fraction thereof, which is discussed on p. 329. There is also said to be some sulphur in basic form, as salts of diethylmethylsulfonium hydroxide.  $(C_2H_5)_2-S(CH_3)OH$ .<sup>94</sup>

We may expect, therefore, to find no increase or change in the urinary sulphur in early tuberculosis, an increase in the neutral sulphur in advanced tuberculosis with cachexia, and an increase in the ethereal sulphur in cases with intestinal lesions in which aromatic products of intestinal putrefaction are increased. Unfortunately we have few accurate studies in patients with measured dietary sulphur. The early investigators reported results in harmony with the preceding statement. Thus,

<sup>93</sup> Berl. klin. Woch., 1902 (39), Nos. 44-47; Deut. Med. Woch., 1902 (28), 697.

<sup>94</sup> Neuberg and Grosser, Zent. f. Physiol., 1906 (19), 316.

Clemens<sup>95</sup> says that Brieger found no increase in the total sulphur in the urine in uncomplicated pulmonary tuberculosis; Hoppe-Seyler found the ethereal sulphur increased in tuberculosis only when there are intestinal complications, and Coggi found a reduced excretion of sulphur in advanced cases with deficient alimentation. Müller failed to find hydrogen sulphide in the urine of consumptives with lung gangrene even when the sputum contained much H<sub>2</sub>S.

Ott<sup>54</sup> investigated the sulphur metabolism in 3 cases of phthisis (see table, p. 293), and reported that each showed a loss of about 0.7 gram sulphur per day, irrespective of whether the N balance was plus or minus. Brasch<sup>55</sup> studied 4 cases in different stages and found that the sulphur balance paralleled the N balance, whether plus or minus. Furthermore, there was no abnormality in the proportion of ethereal and total sulphur. The results of Ott he attributes to faulty analytical methods. Although this is probably correct as to the total sulphur balance in tuberculosis, later observations have not corroborated Brasch as to the proportion of neutral sulphur. Normal human urine on mixed diet contains from 0.10 to 0.24 gram neutral sulphur per day, averaging in the analyses of Weiss<sup>96</sup> about 0.156 gram per day, and constituting an average of 16.5 per cent of the total sulphur. While the urinary sulphur comes both from the food and from the tissues, a relatively large proportion of the neutral sulphur comes from the tissues, and hence the proportion of neutral-S increases in diseases with tissue destruction. Therefore Weiss found that, in common with other wasting diseases, in tuberculosis the urinary output of neutral-S is increased absolutely, and also relatively in proportion to the total sulphur, although the highest figures were obtained in cancer cachexia. He found the average in pulmonary tuberculosis to be 0.1737 gram neutral-S per day, or 20.9 per cent of the total sulphur, and his average figures for different stages are given in the following table.

<sup>95</sup> Ott's Chem. Path. d. Tuberc., p. 189.

<sup>96</sup> Biochem. Zeit., 1910 (27), 178.

| DIAGNOSIS                       | STRENGTH OF DIAZO-REACTION | NUMBER OF DETERMINATIONS | TOTAL S | NEUTRAL S | NEUTRAL S IN PER CENT OF TOTAL S |
|---------------------------------|----------------------------|--------------------------|---------|-----------|----------------------------------|
| Early pulmonary tuberculosis    | Negative                   | 5                        | 0.6977  | 0.1256    | 18.0                             |
| Advanced pulmonary tuberculosis | Positive 2                 | 6                        | 0.8159  | 0.1485    | 18.2                             |
| Advanced pulmonary tuberculosis | Positive 3                 | 8                        | 0.9328  | 0.1875    | 20.1                             |
| Advanced pulmonary tuberculosis | Positive 4                 | 8                        | 0.8215  | 0.1791    | 21.8                             |
| Advanced pulmonary tuberculosis | Positive 5                 | 3                        | 0.8546  | 0.2282    | 26.7                             |
| Carcinoma                       | Two cases both positive    | 5                        | 0.5416  | 0.1598    | 29.5                             |

In general, the more extensive the tuberculosis, the higher the fever, and the worse the prognosis, the higher the neutral-S values, and the Ehrlich diazo reaction parallels them in intensity. Weiss also found that on the average 68.4 per cent of the neutral sulphur was precipitated in the oxyproteic acid fraction of the urinary nitrogen, which is increased parallel to the neutral sulphur. He therefore concludes that the proportion of neutral sulphur, and the proportion of oxyproteic acid-N, are equally a measure of protein destruction, part coming from the food and part from the tissues. In the urochrome precipitate (by copper acetate after Dombrowski) from the oxyproteic acid fraction of the urine was found but about 10 to 20 per cent of the neutral sulphur, as shown in the following table.

*Urochrome (according to Dombrowski)*

| DIAGNOSIS                                | TOTAL S | NEUTRAL S | TOTAL S | URO-     | NEU-     | URO-   |
|--|---------|-----------|---------|----------|----------|--------|
|  |         |           |         | CHROME S | per cent | TRAL S |
| Normal.....                              | 0.9784  | 0.1888    | 19.3    | 0.0394   | 20.8     | 0.788  |
| Pulmonary tuberculosis, first stage..... | 0.8442  | 0.1907    | 22.5    | 0.0294   | 15.4     | 0.588  |
| Pulmonary tuberculosis, third stage..... | 1.1082  | 0.2797    | 25.2    | 0.0269   | 9.6      | 0.538  |
| Carcinoma, stomach.....                  | 0.4837  | 0.1504    | 31.0    | 0.0173   | 11.5     | 0.346  |
| Pulmonary tuberculosis, third stage..... |         |           |         | 0.0429   |          | 0.8576 |

It is possible that in advanced phthisis reduced oxidation may be a factor in raising the proportion of unoxidized sulphur, for Zugsmith and Kahn<sup>97</sup> found an increase in asthma, as well as a lowered creatinine output, which also indicates reduced oxidation.

#### *Chloride metabolism<sup>98</sup>*

As the chlorides enter and leave the body in the same form, they take no part in the actual metabolism of the body. While most of the chlorine is in the form of the sodium salt, nearly one-half as much of the urinary chlorine is united with potassium, and small amounts with ammonia and magnesia. The proportion of potassium to sodium salts in the urine increases with starvation, both because there is no sodium chloride ingested and because potassium occurs in relatively greater amounts in the body cells, hence with tissue destruction there is a relatively greater loss in the urine. Thus, R. Meyer<sup>99</sup> found in a few cases of cancer and advanced phthisis a greatly reduced base excretion and a change in proportion of K to Na to 2:1 or even 3:1. In early cases the normal excess of sodium was found. In all studies of this kind it is necessary to consider the losses in feces, sweat and sputum, and to analyze accurately the diet, since some foods, especially vegetable, are much richer in potassium than others; these precautions have not generally been taken. Mayer's tables (p. 294) based on such analyses (except for consideration of sweat and sputum) show a marked decrease in the total chloride elimination, with a relatively increased excretion of potassium and diminished sodium excretion, with some apparent retention of potassium and more of sodium. These results agree with the expectations and are probably correct.

Meyerowitsch<sup>100</sup> studied the chloride and water balance in 4 cases of febrile pulmonary tuberculosis and found that when the course of chloride metabolism was followed in twelve or twenty-four hour periods, within such brief periods a chloride balance does not strictly exist, for sudden increases in ingested NaCl are fol-

<sup>97</sup> Arch. Int. Med., 1918 (21), 510.

<sup>98</sup> See Host, Jour. Lab. Clin. Med., 1920 (5), 713; Myers, ibid., 1920 (6), 17.

<sup>99</sup> Deut. Med. Woch., 1901 (27), 625.

<sup>100</sup> Nadeschda Meyerowitsch, "Ueber den Kochsalzstoffwechsel bei fiebernder Tuberkulose" Inaug. Dissert., Zürich, 1911.

lowed by retention of various degrees, which are more marked and persistent when the body shows an increased need of water or when there has been a previous chloride loss. Both water and chloride excretion decrease with a rise of temperature; this may occur, even after renewed ingestion of NaCl, without appreciable change in the concentration of the urine. The largest excretion of salt occurs regularly with the temperature remission, when the urine shows the greatest concentration. Pulmonary tuberculosis has an influence on NaCl metabolism in so far as the latter is dependent on the water economy of the organism; in this connection perspiration and frequency of respiration are of determining importance.

Chloride retention in fever, inflammatory exudations and edema, has been known for seventy years, and the subject of much study in recent times. Whether the water retention or the salt retention is primary, and the extent to which the kidneys are concerned in the retention, are still unsettled matters. We know of no recent accurate studies on the chloride balance in tuberculosis except that of Meyerowitsch, but it is to be assumed that retention occurs under the same conditions as in other diseases; that is, when there are acute pneumonic complications, or during the formation of large serous effusions, or when there is edema. The extensive renal involvement often seen in pulmonary tuberculosis in the form of the large white kidney with marked changes in the tubular epithelium, is the anatomical type with which one might expect to get marked chloride retention.<sup>101</sup> However, even with marked terminal or cachectic edema in phthisis, the kidneys may show no anatomical or functional change sufficient to account for the edema.<sup>102</sup>

Kindborg<sup>103</sup> found that in early stages of renal amyloidosis with tuberculosis, the serum chlorine figure may be found low despite a normal urinary excretion of Cl, as if the kidneys had an increased capacity for excreting Cl, which he attributes to a form of hypertrophy of the cells of the convoluted tubules. Boenheim<sup>104</sup>

<sup>101</sup> See Borchardt, Deut. med. Woch., 1912 (38), 1723.

<sup>102</sup> Duboff and Markel, Amer. Review Tuberc., 1922 (5), 973.

<sup>103</sup> Études sur le Rein des Tuberculeux, Paris, 1913, G. Steinheil.

<sup>104</sup> Beitr. Klin. Tuberk., 1921 (49), 233.

has found that when there are no renal lesions there may be some retention of chlorides in severe cases, despite a tendency to hypochloremia, suggesting the possibility of tissue fixation of chlorides.

#### RÉSUMÉ

We can have little accurate knowledge concerning the changes in metabolism induced by tuberculosis *per se*, for seldom do we have under investigation a tuberculous patient who does not suffer from some complication, especially mixed infection, which influences the metabolic change. Acute pneumonic tuberculosis before secondary infection has taken place, acute generalized miliary tuberculosis without meningitis, and systemic tuberculin reactions in uncomplicated cases of tuberculosis, give us the best illustrations of the influence that tuberculous infection itself may have on metabolism. But for the purposes of the clinic we do not care so much to know exactly what part of any observed metabolic effects depend on the tubercle bacillus alone—we are concerned with the metabolism of the phthisical patient with such complications as he ordinarily presents. Recognizing these factors, we may recapitulate the evidence brought out in the preceding chapter and the immediately following chapter on urine in tuberculosis, as follows:

Basal metabolism in tuberculosis without fever is little altered from the normal figure. If reckoned on the basis of the surface area actually presented by the patient's body, it is commonly a little increased above the normal figures per square meter; but if compared with the surface area presented by the same individual before he became emaciated by the disease, the heat production is usually equal to or less than the estimated calories per hour for the same patient. That is, the actual heat production of the afebrile consumptive is about the same as in the normal person, but as he emaciates, heat production does not always decrease *pari passu* with the decreased surface area of the body.

As with other diseases, increased body temperatures usually increase the heat production, but this increase may be less in a cachectic consumptive than it would be in a robust person with a correspondingly high temperature. The patient with severe pulmonary tuberculosis generally shows an increase of 20 to 40

per cent in the metabolism calculated from the body surface; with high fever the increase may be 50 to 75 per cent. In early tuberculosis the caloric requirement is about 1000 to 1200 per square meter of body surface, but with fever the requirement may be 1500 calories or even a little more.

The respiratory quotient in tuberculosis is usually found within the normal limits, indicating that there is no noteworthy change in the proportion of protein and non-protein combustion in tuberculosis.

The specific dynamic action of protein manifests itself the same in tuberculosis as in health.

Reduction in lung volume, which is an important feature of pulmonary tuberculosis, cannot be shown directly to lead to a reduced oxidation, for studies on man and animals with greatly reduced amounts of lung tissue do not show any recognizable interference with oxidation in the body as a whole. Nevertheless it would seem probable that some deficiency must result from the lung involvement in advanced cases, in favor of this view being the prominence of fatty changes in the viscera in phthisis.

The total capacity of the lungs is decreased in proportion to the amount of involvement, and the vital capacity varies with the changes in the progress of the disease. Residual air is ordinarily much less affected than either the vital capacity or the total capacity.

Although there is some evidence of decreased gastric motility and secretory activity in phthisis, and also low pancreatic activity, the alimentary tract seems able to handle in an entirely normal manner even excessive quantities of food. Digestion and assimilation are usually entirely adequate in the tuberculous, whether febrile or afebrile, unless there are definite intestinal lesions. With intestinal ulceration, however, large amounts of nitrogen may be lost in the feces. Fat diarrhoea has also been observed in patients with intestinal amyloidosis, and when the lymphatics are blocked through tuberculosis of the glands.

In some cases there is enough loss of protein in the sputum to constitute a really serious drain on the nutrition. While there may be appreciable excretion of nitrogen in the sweat, this is mostly in the form of urea and does not indicate tissue loss, but is merely a source of error in studies of the nitrogenous metabolism in tuberculosis.

The metabolism of the febrile tuberculous subject probably follows the same laws as in fever from other causes, most of the heat being derived from carbohydrates, fat destruction being increased from chills and increased muscular activity, tissue protein destruction resulting only from toxic influences and not from the fever per se. Because of the existence of intoxication in tuberculosis the minimal nitrogen excretion on an abundant carbohydrate diet with low protein, is much higher than the normal "wear and tear" nitrogen excretion of a healthy man (5 to 6 grams N per day in the tuberculous as against 2.5 to 4 grams N per day in the normal subject).

It seems possible to maintain nitrogen balance with 60 to 70 grams protein per diem in many cases of tuberculosis, although some cases will require more. Since the specific dynamic action of protein leads to increased pulmonary work, it would seem desirable not to exceed too greatly the protein supply beyond the actual needs in active febrile progressive cases.

There is abundant evidence of a toxicogenic destruction of protein in tuberculosis. Early, afebrile cases may show practically a balance of nitrogenous intake and excretion, but even here there is probably some toxic tissue destruction, since these patients on a liberal nitrogen supply, being more or less emaciated, should properly show considerable retention of nitrogen. Febrile cases almost always show a distinct loss of nitrogen, most marked in acute caseous pneumonia, but not always in direct proportion to the amount of fever. As a similar toxicogenic loss of tissue protein is observed to result from febrile tuberculin reactions it may be assumed that tuberculosis may produce this same effect independent of secondary infections. Probably the poisons in tuberculosis act both by increasing the tissue destruction and by interfering with tissue regeneration.

Other evidences of toxicogenic destruction of tissue protein are observed in tuberculosis.

1. The purine nitrogen excretion is increased, both in active tuberculosis and during febrile reactions to tuberculin. The fact that purine bases are increased relatively more than uric acid suggests interference with tissue oxidation.

2. Creatinine excretion increases with increase in disease activity up to a certain point, when reduction in muscle volume lowers the output.

3. The proteic acid or colloidal-nitrogen fraction of the urine is greatly increased. This seems to be derived entirely from and in proportion to the tissue destruction.

4. The proportion of organic phosphorus to total phosphorus in the urine is increased, indicating disintegration of nucleoproteins and lecithin.

5. The proportion and also the total quantity of neutral sulphur in the urine is also increased.

6. The proportion of potassium to sodium in the urine is increased, the former being largely derived from the tissues.

7. Albumosuria is often seen in tuberculosis, and as it also appears during systemic tuberculin reactions, it is probably derived from tissue destruction.

Despite the widespread belief that tuberculosis is characterized by a marked loss of inorganic elements, "demineralization," there is no evidence for such a process in carefully controlled observations on mineral metabolism. At most the losses of salts are no greater in tuberculosis than in other conditions with a corresponding loss of weight. Calcium, which has been especially considered in tuberculosis, seems not to suffer any particular loss, and the blood content of calcium is normal. We cannot find satisfactory evidence that silica metabolism is altered in tuberculosis. The figures on phosphorus metabolism do not indicate any noteworthy loss of this element, but the proportion of the urinary phosphorus in organic combination seems to be somewhat increased, presumably from increased nucleoprotein disintegration.

The constant presence of more or less degenerative change in the renal epithelium is presumably part of the toxic tissue destruction of tuberculosis. This is accompanied by marked albuminuria in but a very small proportion of the cases, and less than is usually seen in more acute intoxications with equal anatomical changes in the kidneys. Mucoid-like compounds of chondroitin sulphuric acid are found more frequently than true protein, although not usually in large amounts and these are believed to come from the injured renal epithelium.

## CHAPTER XII

### THE URINE IN TUBERCULOSIS

Many of the changes in the urine have already been discussed in the preceding section on the metabolism in tuberculosis. In the following chapter we consider further the urinary changes found in tuberculosis, most of which have a distinct relation to the metabolism.

#### PHYSICAL CHARACTERS

The quantity and specific gravity of the urine in tuberculosis present no features peculiar to the disease. Obviously when there is a drenching perspiration the amount of urine is reduced and when there is a high fever a concentrated urine will be excreted. Polyuria has been described by older writers as a "pretuberculous" sign, but is now not recognized as such. Amyloidosis may lead to polyuria, at least in its early stages. The more common parenchymatous nephritis may result in a reduction in the amount of urine, with or without dropsy, and oftentimes a vicarious excretion of fluid through the bowels may reduce the amount of urine. Nevertheless, it seems to be true that in very early tuberculosis, polyuria is not infrequently seen, and in late stages oliguria from sweating, fever, diarrhea, and sometimes from deficient fluid intake in patients not under proper care. During a tuberculin reaction there may be a sharp polyuria if absorption of serous effusion results therefrom, otherwise there may be no change or either polyuria or oliguria.<sup>1</sup>

#### *Reaction*

Likewise there is nothing significant in the reaction of the urine, which, according to most observers, varies under the same influences as in other conditions. Blumenthal says that during

<sup>1</sup> Neumann, Beitr. Klin. Tuber., 1918 (39), 187; Kirch, ibid., 1921 (47), 429.

resorption of exudates there is an increased alkalinity of the urine. Hale White reported that a protracted high acidity of the urine, drawn from the body and left standing, is of diagnostic significance in tuberculosis,<sup>2</sup> and maintained that the acidity of fresh urine from patients with pulmonary tuberculosis is usually greater than that from patients with other diseases, irrespective of concentration. No lactic acid could be found to account for this, and no excess of phenols or other recognizable antiseptic substances to explain why the urine in tuberculosis remains so long acid and free from decomposition, although the tuberculous urine showed some evidence of increased bactericidal power.

#### *Surface tension*

Among the many substances that compose the non-protein colloidal fraction of the urine, are colloids or semi-colloids of different degrees of dispersion which diminish the surface tension of the urine, and hence are called "stalagmones" by Bechhold and Reiner.<sup>3</sup> These authors state that stalagmones exhibit particular characteristics in certain diseases, including tuberculosis, in which the surface tension of the urine is usually lowered. In general the lowering of surface tension parallels the amount of color in the urine, although it probably depends not on urochrome but on albumoses, oxyproteic acids, etc. Protein and bile have a similar effect. Schemensky<sup>4</sup> uses a surface tension quotient to express the alteration in surface tension of the urine, and finds that this quotient, which rises with the amount of stalagmones, gives information of value concerning the prognosis and progress of phthisis.

#### *Freezing point*

Cryoscopic study of the urine merely indicates the number of molecules and ions in solution, and gives a more accurate picture of its concentration than chemical analysis. Naturally the urine

<sup>2</sup> See Hale White, Brit. Med. Jour., 1892, May 21, p. 1070; White and Janmahomed, Quart. Jour. Med., 1909 (2), 397; Barabaschi, Gaz. degli Osped., 1912 (32), 123.

<sup>3</sup> Münch. med. Woch., 1920 (67), 891; Biochem. Zeit., 1920 (108), 98.

<sup>4</sup> Zeit. klin. Med., 1922 (93), 334.

of high specific gravity is usually the one with the greatest increase in the depression of freezing point,  $\Delta$ , and such urines are usually found with fever or sweating. Tuberculosis exhibits no peculiarities in respect either to the molecular concentration or to the ionic concentration, which latter determines the degree of electrical conductivity.

#### NITROGEN CONTENT

The composition in respect to urea and the other ordinary constituents offers nothing striking. The details of urinary change as part of the picture in metabolism in tuberculosis are discussed to a large extent in the preceding chapter.

As giving the general features of the composition of the urine in tuberculosis we may cite the figures reported by Labb  and Vitry,<sup>5</sup> obtained by averaging the analytical results from the urines of 21 patients with advanced pulmonary tuberculosis. Several of these were in the last days of life and the figures obtained in these cases were compiled separately from those not quite so near the end.

|                             | ADVANCED      | PREMORTAL     |
|-----------------------------|---------------|---------------|
| Quantity.....               | 962.0 cc.     | 817.0 cc.     |
| Total N.....                | 8.48 grams    | 7.44 grams    |
| Urea.....                   | 15.16 grams   | 13.30 grams   |
| Urea N:Total N (ratio)..... | 81.8 per cent | 80.1 per cent |
| Purines.....                | 0.68 gram     | 0.686 gram    |
| Purine N:Total N.....       | 2.5 per cent  | 3.1 per cent  |
| Ammonia.....                | 0.77 gram     | 0.79 gram     |
| Ammonia N:Total N.....      | 6.0 per cent  | 8.9 per cent  |
| Undetermined N.....         | 0.61 gram     | 0.62 gram     |
| Undetermined N:Total N..... | 6.4 per cent  | 8.7 per cent  |
| Amino N.....                | 0.08 gram     | 0.129 gram    |
| Amino N:Total N.....        | 1.0 per cent  | 1.7 per cent  |

Considering these figures, we find that the urinary excretion of nitrogen was low, because of the low intake of food by these very ill patients, and despite some excessive tissue breakdown. The latter is evident from the fact that the ratio of urea-N to total-N was less than normal, because of an increased proportion of purine,

<sup>5</sup> Revue de m d., 1912 (32), 818.

ammonia, amino and undetermined nitrogen, especially in the terminal stages. Other analyses of the urine in tuberculosis might be cited, but the general features would correspond to those given above. The relation of the blood nitrogen to that of the urea excretion as expressed in the Ambard coefficient also fails to show any significant features in tuberculosis, although in very active cases with renal injury, it may be increased, even this finding not being constant (Weil<sup>6</sup>). In very advanced cases the coefficient may be lowered.

#### CARBON EXCRETION

The proportion of carbon to nitrogen in the urine varies directly with the amount of colloidal-N or the proteic acid fraction, and less than half the urinary carbon is in the form of urea. It is fairly constant in normal persons, the relation  $\frac{C}{N}$  being from 0.7 to 1.0, and this figure is not appreciably altered by the diet.<sup>7</sup> With febrile conditions and after severe exercise the proportion of carbon is raised, but Steyrer<sup>8</sup> did not find any change during the tuberculin reaction, although previously Scholz<sup>9</sup> had reported an increased carbon ratio under the same condition. It is to be assumed, however, that the carbon ratio will fluctuate in much the same way as the proteic acid fraction.

#### *The urine of tuberculous animals*

The chemistry of the urine of an experimental animal with tuberculosis may be of some scientific interest, and hence we publish the following findings of Dehaussy.<sup>10</sup> Rabbits of approximately 3 kgm. weight maintained in nutritive equilibrium on a regimen of 150 grams sugar beets and 150 grams oats daily were used as experimental animals. The volume of the urine varied only to a slight extent in general, but pronounced polyuria was observed during the days immediately preceding death. Hyperchloruria was observed at the beginning of infection, but, with the approach of death, the chloride value decreased to considerably below normal (0.036 gram per twenty-four hours as compared with the normal value 0.170 to 0.200 gram).

<sup>6</sup> Revue de la tuberc., 1921 (2), 110.

<sup>7</sup> Magnus-Alsleben, Zeit. klin. Med., 1909 (68), 358.

<sup>8</sup> Zeit. exp. Path., 1907 (4), 709.

<sup>9</sup> Arch. exp. Path. Pharm., 1898 (40), 326.

<sup>10</sup> Compt. Rend. Soc. Biol., 1914 (77), 124.

The phosphate value was greatly increased fifteen days after inoculation (0.600 gram as compared with the normal value 0.150 to 0.175 gram); ten days later it dropped to 0.070 gram and this hypophosphaturia was followed by a slight increase at the moment of death. Elimination of uric acid was greatly increased at the beginning of infection (0.038 gram as compared with the normal value 0.010 to 0.014 gram); the uric acid value then decreased progressively to a minimum of 0.008 gram immediately before death. The amount of nitrogen eliminated showed a pronounced increase at the beginning of infection; it then decreased below normal and finally increased considerably above normal four to five days before death. Intense calciuria, which rapidly decreased, was observed in the second week following infection; increased elimination reappeared, but with less intensity, at the approach of death. Tests for glucose were invariably negative. Albuminuria appeared in a few days after inoculation and persisted until death. The Moritz-Weiss reaction was applied to fresh urine and was always found to be negative.

#### PROTEIN EXCRETION

##### *Albuminuria*

In tuberculosis a greater or less amount of renal injury is common, and in advanced stages this may be an important factor. Flick<sup>11</sup> says "clinically nephritis is one of the complications of pulmonary tuberculosis most to be dreaded." Most commonly the lesions are of the tubular type, and may or may not be severe enough to cause demonstrable effects. Occasionally we have both the anatomical and clinical picture of the typical chronic parenchymatous nephritis with the large pale, lipoid-rich kidney. Amyloidosis, when present, is a serious complication of tuberculosis. Walsh<sup>12</sup> has studied the kidney carefully in 100 consecutive autopsies, finding in all greater or less anatomical change, which he classifies as follows:

|                                       |     |
|---------------------------------------|-----|
| Acute parenchymatous nephritis.....   | 36  |
| Chronic parenchymatous nephritis..... | 33  |
| Diffuse nephritis.....                | 4   |
| Interstitial nephritis.....           | 14  |
| Amyloid degeneration.....             | 5   |
| Cloudy swelling.....                  | 7   |
| Passive congestion.....               | 1   |
|                                       | 100 |

<sup>11</sup> Trans. Natl. Assoc. Tuberc., 1905 (1), 198.

<sup>12</sup> Trans. Natl. Assoc. Tuberc., 1906 (2), 205.

In these 100 cases of tuberculosis with renal changes Walsh found albuminuria present in nearly half, but generally only as a trace. In another series he found distinct amounts of albumin only twice in 44 cases of tuberculosis with tubercle bacilli in the urine. Casts in small numbers may be found in the majority of cases. Tubercle bacilli were present in 82.5 per cent of cases of tuberculosis without frank renal tuberculosis, although over 50 per cent of the kidneys in pulmonary tuberculosis show at least microscopical tubercles if examined thoroughly.<sup>13</sup>

The urinary changes resulting from the renal damage of tuberculosis are in no appreciable way different from those observed in similar renal lesions from other causes, and will not be discussed here, beyond quoting the remark of Flick that "One of the remarkable features of nephritis in tuberculosis is the non-elimination of albumin. Even when the kidneys are extensively diseased there is very little albumin in the urine, and sometimes there is none." Also we recall the statement by Ott that the so-called Mörner's protein, which is a mucoid-like compound of protein with chondroitin-sulphuric acid, is more or less increased in tuberculosis, its significance not being exactly known, but it is now generally considered to be an indication of slight but actual damage to the renal tissues from the poisons of the disease.<sup>14</sup>

Some authors have spoken of a "pretuberculous albuminuria," indicating that tuberculosis suspects often show albuminuria for some time before the tuberculosis can be demonstrated. There was once a tendency to attribute this to a phosphaturia or an oxaluria. Presumably it is merely the result of the toxic injury to the kidney during the allergic reaction which occurs when re-infection of the already tubercularized subject is overcoming the resistance. D'Onghia<sup>15</sup> discusses at length the albuminuria of tuberculosis, which in greater or less degree he finds to be almost constantly present, increasing with disease, and after exercise, fever, and during digestion. With exacerbations appear casts in the urine, mostly hyaline but sometimes with granules and lymphoid cells attached. Very probably the slight albuminurias

<sup>13</sup> See also Kieffer, Zeit. f. Tuberk., 1920 (33), 9.

<sup>14</sup> See Pollitzer, Med. Klin., 1914 (9), 2106.

<sup>15</sup> Beit. Klin. Tuberk., 1914 (29), 409.

without casts are to be considered as orthostatic albuminuria, to which the tuberculous are especially prone,<sup>16</sup> and which some look upon as a definitely pre-tuberculous phenomenon.

The terminal acute nephritis that is sometimes seen, even of a hemorrhagic type, may well represent an allergic reaction, as suggested also by Kindborg.<sup>17</sup>

Although the foregoing and other statements in the literature might give the impression that albuminuria is frequent in tuberculosis, nevertheless Flick's statement as to the relatively small amount of albumin excretion holds good, for the amounts found in these positive cases are usually extremely small. Dietl<sup>18</sup> states that in 175 severe cases of pulmonary tuberculosis he found a positive reaction for coagulable protein in but 11.4 per cent although there was a positive reaction for protein precipitated with acetic acid, that is, Mörner's chondroitin-acid protein, in 42.8 per cent and a positive reaction for chondroitin sulphuric acid was given by 92.8 per cent. Lichtweiss found albumin in 32 of 100 cases but in only 5 was there a strong protein reaction. Dietl finds the detection of chondroitin sulphuric acid of little prognostic value unless it is persistent, and notes that this material may appear in advance of albuminuria. The chondroitin-sulphuric-acid protein compound he found chiefly in cases with a bad prognosis but even this is of little significance. He likens the significance of chondroitinuria to that of urobilinuria, the former merely signifying that the poisons of the disease have injured the kidneys while the urobilin indicates that the liver has suffered. Each is a sensitive test for injuries of the respective organs. However, in a later article, Dietl<sup>19</sup> reports finding chondroitin-sulphuric acid in advance of albuminuria in cases of renal amyloidosis, and suggests that especially in cases of bone tuberculosis chondroitinuria should be considered an indication of beginning renal amyloidosis.

<sup>16</sup> Hinze and Sorin, Beitr. Klin. Tuberk., 1912 (24), 255.

<sup>17</sup> Études sur le rein des tuberculeux. G. Steinheil, Paris, 1913. Also see Müller-Deham and Kothny, Wien Arch. inn. Med., 1921 (2), 509.

<sup>18</sup> Wien. klin. Woch., 1921 (34), 133.

<sup>19</sup> Beitr. Klin. Tuberk., 1922 (51), 18.

*"Albumosuria"<sup>20</sup>*

If proteoses<sup>21</sup> enter the blood stream they appear in large part in the urine, indicating that the tissues do not readily utilize them in this form.<sup>22</sup> Consequently, when proteoses are produced in considerable amounts by autolysis of pathological tissues they appear in the urine and their presence is considered to be of diagnostic value. True peptone seems rarely, and according to many observers never, to appear in the urine; but in view of the observations that polypeptides often appear in the urine,<sup>23</sup> it is probable that true peptones also do.<sup>24</sup> "Albumose," therefore, may be found in the urine whenever any considerable amount of tissue or exudate is being autolyzed and absorbed, and it has been found in the following conditions: Suppuration of all kinds; resolution of pneumonia; involution of the puerperal uterus; carcinoma (two-thirds of all cases—Ury and Lilienthal) and other malignant growths; febrile conditions with tissue destruction (37.5 per cent of all cases—Morawitz and Dietschy);<sup>25</sup> acute yellow atrophy, phosphorus poisoning and eclampsia; leukemia, especially under x-ray treatment; absorption of simple and inflammatory exudates; ulcerating pulmonary tuberculosis, osseous tuberculosis, and after tuberculin reactions. In ulcerative conditions of the alimentary canal, albumoses may be absorbed unchanged and cause alimentary albumosuria. The normal kidney seems to be impermeable to the small amounts of proteose that may be present normally in the blood, or even after large oral

<sup>20</sup> Critical review given by Pollak, *Zeit. exp. Med.*, 1914 (2), 314.

<sup>21</sup> The term proteose is preferable to albumose as being more general, but usage has fixed the term albumosuria, and hence it is accepted in this discussion.

<sup>22</sup> They may be partly hydrolyzed into smaller complexes, however, primary proteoses being partly changed to deutero-proteoses, and the latter partly to peptones (Chittenden, Mendel, and Henderson, *Amer. Jour. Physiol.*, 1899 (2), 142).

<sup>23</sup> Chodat and Kummer, *Biochem. Zeit.*, 1914 (65), 392.

<sup>24</sup> Richaud (*Jour. pharm. Chim.*, 1921 (23), 376) reports that peptonuria is more frequent than albumosuria, finding it in 36 of 71 specimens of pathological urine of which only 4 showed albumose.

<sup>25</sup> *Arch. exp. Path. Pharm.*, 1905 (54), 88.

ingestion of proteoses, but in parenchymatous nephritis it may escape in the urine (Henderson,<sup>26</sup> Pollak<sup>20</sup>).

As has been stated in the discussion of the chemistry of tuberculous lesions, these are ordinarily characterized by the extremely slow rate of autolysis, and hence there is probably seldom if ever sufficient proteose or peptone liberated from them to produce a recognizable albumosuria. When acute suppuration of tuberculous lesions takes place, however, or when toxicogenic destruction of the body protein occurs in sufficient degree, demonstrable amounts of albumosuria may be found.

It is possible that some of the symptoms of these conditions are due to intoxication with proteoses, for 0.07 to 0.1 gram deutero-albumose will cause a febrile reaction in a healthy man<sup>27</sup> but probably their amount is usually too small to cause appreciable effects.<sup>28</sup> It is well known, however, that the characteristic rise of temperature following the injection of tuberculin into tuberculous individuals is also produced if minute quantities of proteose solutions are injected in place of tuberculin; therefore, proteoses arising from autolysis in tuberculosis may be of importance in causing fever and other symptoms,<sup>29</sup> and possibly also renal injury.

Clemens reviews at length the early literature on the presence of albumose and peptone in the urine in tuberculosis,<sup>30</sup> which was the subject of much attention in the early days of the clinical laboratory. He recognizes that these substances may appear in the urine either through the disintegration of large quantities of tissue or exudate or from an enterogenous source when the loss of mucous membrane permits absorption directly into the blood from the intestines, several writers having described "peptonuria" in intestinal tuberculosis. The literature on this subject goes back as far as 1879 when Maixner found so-called peptonuria in cases of phthisis with cavities which were not draining. Von

<sup>26</sup> Lancet, Mar. 6, 1909.

<sup>27</sup> See Matthes, Arch. exper. Path. u. Pharm., 1895 (36), 437.

<sup>28</sup> In a series of unpublished experiments I was unable to cause amyloid degeneration in rabbits by protracted intoxication with proteose solutions.

<sup>29</sup> Simon, Arch. exp. Med., 1903 (49), 449. Concerning relation of tuberculin to proteoses see review by Jolles in Ott's "Chemische Pathol. der Tuberkulose," also Chapter II of this book.

<sup>30</sup> Ott's Chem. Path. Tuberk., p. 238.

Jaksch found it in tuberculosis only when purulent infiltration was occurring. Pacanowsky observed "peptonuria" in 11 of 25 cases of pulmonary tuberculosis, mostly when the physical symptoms were not well marked. On the other hand some authors obtained no positive reactions in tuberculosis, the results apparently depending on the method employed. Stadelmann gave a critical review of the literature in 1894, since when the term of albumosuria has been generally used. The later literature shows that albumosuria does occur not rarely in tuberculosis, even without fever, the quantity of albumoses found apparently not being large enough to produce fever in these individuals. It has been observed that albumosuria is likely to accompany the Ehrlich diazo reaction. Ott found that consumptives who develop a fever from exercise often show at the same time small amounts of albumose in the urine, although there had been none in the afebrile stage, an observation which other observers have not confirmed. Following subcutaneous injection of tuberculin, albumosuria has been observed occasionally; in 33 of 300 cases studied by Kahler. Similar results have been reported by several others.

Much of the earlier work on albumosuria cited by Clemens was inaccurate because of defective methods and unconsidered sources of error, of which Pollak<sup>20</sup> mentions: (1) Urobilin gives the biuret reaction; (2) urine contains, even under normal conditions, protein substances that may not be readily removed, and hence come down in the albumose fraction (Mörner's urine proteins, mucoids, nucleins, etc.); (3) protein escaping precipitation by heat through the formation of acid albumin; (4) globin from hemoglobin in bloody urine, this protein being difficult of coagulation; (5) artificial formation of albumoses by hydrolysis during the removal of protein by heat; (6) entrance of albumoses from lesions in the lower urinary tract, and of albumose-like substances found in sperm and prostatic secretions; (7) formation of proteoses through autolysis of the urine standing in the bladder or after its collection.

Nevertheless, despite all these possible sources of error<sup>31</sup> the general facts determined by early workers stand today as correct.

<sup>31</sup> See also Dietschy, Beitr. Klin. Tuberk., 1912 (24), 279.

We still recognize that true albumosuria does occur, and that the albumose may have the following origins: (1) Endogenous, from autolysis in inflammatory lesions, absorption of exudates or dead tissue, excessive catabolism of tissue proteins. (2) Enterogenous or alimentary, by direct absorption of albumose through an abnormal bowel wall. (3) Renal, through increased permeability of the kidneys for albumoses present in the blood in either normal or increased amounts (Pollak).<sup>20</sup>

It is evident that in tuberculosis all three of these possibilities exist, and hence the establishment of albumosuria as a not uncommon condition in tuberculosis has not been difficult. Recent observations have added little of importance to the literature cited by Clemens. We note that Pollak, feeding large quantities of albumose to investigate the urinary excretion thereof, was able to produce "renal albumosuria" in several patients with pulmonary tuberculosis accompanied by renal lesions, whether parenchymatous nephritis or amyloidosis. Also that Morawitz and Dietschy<sup>25</sup> found spontaneous albumosuria in 2 of 8 cases of pulmonary tuberculosis. Deist,<sup>32</sup> using methods that are not absolutely reliable (Dietschy) found positive results in 100 per cent of third stage cases, even when without fever; in early afebrile cases only 20 per cent were positive. Tuberculin reactions he found to cause albumosuria in any stage of the disease, whether with or without rise in temperature. Wolff,<sup>33</sup> using a new method, gives the following statement: If a higher residual-N value is obtained in blood analyses with metaphosphoric acid than with phosphomolybdic acid, the difference may be attributed to albumose-like substances. Using this method in 6 cases of advanced pulmonary tuberculosis with fever, 3 showed an increased albumose value for the blood (10 to 15.5 mgm. N per 100 grams, normal being 1 to 7.5 mgm.) associated with albumosuria, while the other 3 cases did not show this symptom despite the fact that 2 of the last 3, as well as all the first 3, had intestinal ulcers.

<sup>22</sup> Beitr. Klin. Tuber., 1912 (23), 547.

<sup>23</sup> Wolff, Erik, Om Förekomsten av Albumosliknande Substanser i Blodet: Inaug. Dissert., Lund, Sweden, 1920.

*Amino acids*

Undoubtedly increased quantities of amino acids and polypeptides are also present under the same conditions as give other evidences of excessive tissue protein destruction, although we know that in many acute toxic diseases with abundant evidence of extensive tissue injury there may be little or no increase in the urinary amino acids.<sup>34</sup> Nevertheless Labb   and Bith<sup>35</sup> found that although normal individuals showed no increase in the urinary amino acids when fed large doses of peptone in hepatic diseases, including atrophic cirrhosis and fatty cirrhosis in tuberculosis, there results an increased excretion of amino acids, presumably because of deficient hepatic deaminization. Labb   and Vitry<sup>5</sup> found an average daily excretion of 0.08 gram of amino-N in a series of cases of advanced pulmonary tuberculosis, and in the urine from a series of cases a few days before death the average was 0.129; as the total nitrogen excretion was low, the ratio of amino-N to total-N was considerably above normal, especially in the premortal cases. The finding of appreciable amounts of leucine and tyrosine in many different sorts of diseases, including 3 cases of phthisis reported by Anderson,<sup>36</sup> has not been repeated by more recent observers.

Zandren<sup>37</sup> investigated the supposed connection between the function of the liver and the presence of amino-acids in the urine to decide whether the latter is due to an increased toxic decomposition of protein, or to injury of the liver cells. In advanced tuberculosis we have a state of toxic decomposition of albumin and often fatty or amyloid infiltration of the liver. In 13 advanced cases without visible changes in the liver the amino-acid nitrogen was 2.37 per cent of the total nitrogen in the urine. In 36 cases there were fatty or amyloid deposits of the liver (proved postmortem), and the amino-acid nitrogen averaged 4.36 per cent. The conclusion is that in tuberculosis a value of amino-acid beyond 3 per cent of the total urinary nitrogen points to a

<sup>34</sup> See review on amino acids by Van Slyke, Arch. Int. Med., 1917 (19), 56.

<sup>35</sup> Rev. de m  d., 1914 (34), 88; Compt. Rend. Soc. Biol., 1912 (73), 210.

<sup>36</sup> Brit. Med. Jour., Sept. 4, 1880, p. 381.

<sup>37</sup> Acta Med. Scand., 1921 (53), 743; Zeit. klin. Med., 1922 (94), 101.

serious diffuse lesion in the liver cells of some sort. Usually such a rise in amino-acid excretion is accompanied by an increase in the urinary ammonia and by a strong reaction for urobilin.

### Lipins

Appreciable amounts of fat in the urine are pathological, either representing excretion of excessive amounts in the blood in lipemia or fat embolism, or arising locally in fatty degeneration of the renal cells. As more than normal amounts of fatty materials are often found in the urine<sup>38</sup> in tuberculosis it is probable that it comes from the fatty degeneration of the renal cells which is so often present in pulmonary tuberculosis.

Labbé and Golgofsky<sup>39</sup> found in the urine in tuberculosis an increase of the total amount of ether extractable substance, part of which was acid directly neutralizable by alkali and probably chiefly hippuric acid; also the unsaponifiable material was about doubled on the average, this presumably being largely cholesterol.

### TOXICITY OF URINE

Of little more than historical interest are the studies on the toxicity of urine in disease, so extensively pursued by the French school forty years ago, and also the search for the ptomaines to which the poisonous action of the tubercle bacilli was attributed. The observation that sometimes the urine in febrile tuberculosis is more toxic to animals than normal, which was reported by several observers, we would now attribute, if we admit the correctness of the experimental observations, to the concentration produced by fever and sweating, and possibly to the increase in the colloidal-N fraction and of potassium which accompanies tissue catabolism. Undoubtedly Albu was correct in his finding that substances precipitable by alkaloidal reagents are present in the urine in tuberculosis as well as in other conditions, but that they have no specific relation to tuberculosis.

There is no question that in active tuberculosis there is urinary excretion of *tuberculin*, or something that gives the typical tuberculin reaction, as shown by the "auto-urine test" of Wildbolz.<sup>40</sup> This test consists of injecting into the skin of the patient a con-

<sup>38</sup> See Rentz, Nederl. Tijdschr. Geensk., 1918 (1), 728.

<sup>39</sup> Compt. Rend. Soc. Biol., 1912 (73), 332.

<sup>40</sup> Corr.-bl. f. Schweiz. Aerzte, 1919 (49), 793.

centrated portion of his own urine, as in the ordinary cutaneous tuberculin test. If he possesses an active focus of tuberculosis a typical tuberculin reaction will be produced. In cases of persons suspected of tuberculosis who are not reactive to tuberculin the urine may be injected into a person known to give a positive tuberculin test, in whom the reaction will be obtained. This urine test has been extensively employed, usually with satisfactory results<sup>41</sup> since it indicates the presence of active tuberculous processes in the body, sufficient to produce enough free reactive substance to lead to its excretion in the urine. The demonstration of the excretion of the potent reactive constituent of tuberculin by the kidneys may be looked upon as explaining the well-nigh universal presence of renal lesions in tuberculosis, since presumably the renal tissues of the tuberculous are allergic to tuberculin quite as much as the skin or conjunctiva, and hence would suffer the same sort of inflammatory reaction to the tuberculin picked out of the blood in passing through them.

While there is, at the time of writing, not any general agreement as to the diagnostic value of the Wildbolz reaction<sup>42</sup> it is certainly obtained frequently enough to justify considering it as evidence that something resembling or corresponding to tuberculin is excreted in the urine in active tuberculosis.

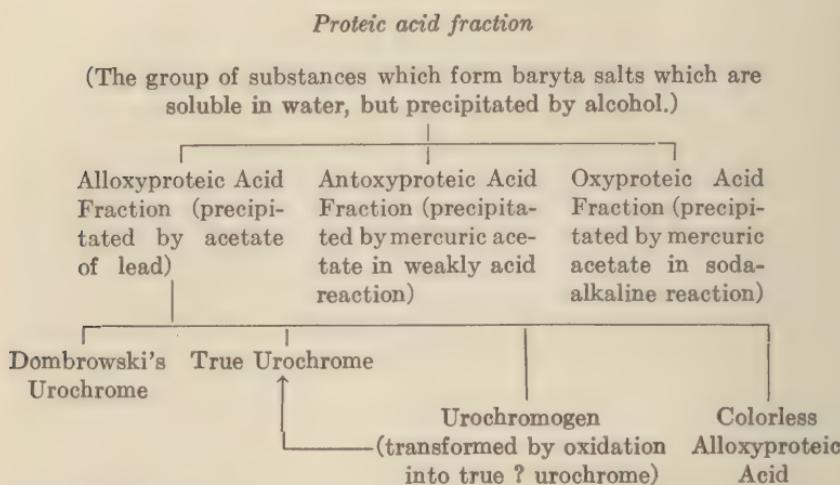
#### PROTEIC ACID

Under this term, or as "oxyproteic acids," is included a mixture of ill defined character, which includes most of the "undetermined-N" of the urine, and also constitutes a large part of the "colloidal-N" fraction. It is distinguished and separated on the basis of the fact that the barium salt is soluble in water but not in alcohol. Although it does not give the biuret test, and tests for the known amino acids are negative, this fraction may perhaps be composed of a mixture of incompletely oxidized polypeptides containing much unoxidized sulphur.

<sup>41</sup> See Gibson and Carroll, Jour. Amer. Med. Assoc., 1921 (76), 1381.

<sup>42</sup> See Bressel, Deut. med. Woch., 1920 (46), 1385; Weiss, Med. Klinik, 1921 (17), 930; Orlansky, ibid., p. 1359; Levi, ibid., p. 1296; Schmid, Schweizer med. Woch., 1921 (51), 996; Grass, Beitr. Klin. Tuberk., 1922 (51), 157; Kuhn, ibid., p. 24.

There are undoubtedly numerous substances included in this fraction, and several sub-fractions have been separated. Fürth<sup>43</sup> gives the following scheme to show the relationship between them:



Whatever the proteic acid fraction may be, one characteristic is that the amount in the urine is generally increased in cachectic conditions, to which tuberculosis forms no exception. Presumably this increase depends upon tissue destruction since a similar increase is observed in phosphorus poisoning, acute febrile diseases and cancer cachexia.

Sassa,<sup>44</sup> who found that normal urine has quite constantly from 4.3 to 4.7 per cent of its nitrogen in the oxyproteic acid fraction, obtained in 5 cases of very advanced phthisis (3 being moribund) the following figures: 5.67, 6.27, 7.65, 5.25, and 3.33.<sup>45</sup> Similar high figures were obtained in cancer with cachexia, but not in early cases. Just when in the progress of phthisis the increase in the oxyproteic fraction of the urine begins, or just what its origin and significance may be, have yet to be determined.

<sup>43</sup> Chemistry of Metabolism, Amer. Edition, 1916, p. 138 (modified from Weiss, Biochem. Zeit., 1911 (30), 338.)

<sup>44</sup> Biochem. Zeit., 1914 (64), 195.

<sup>45</sup> (With but 350 cc. urine in twenty-four hours.)

*The sulphur of the proteic acids*

Salomon and Saxl<sup>46</sup> have introduced a test based on the presence in the urine of a sulphur-containing organic constituent, believed by them to be sulphocyanate, the sulphur of which can be split off by means of hydrogen peroxide, oxidized and determined as barium sulphate. Most of the urochromogen sulphur seems to be included. This material is found in many carcinoma cases, rarely (6 of 182) in normal urines, but also in many cases of tuberculosis. Kahn<sup>47</sup> states that this test is generally negative when the neutral sulphur in the Salomon-Saxl fraction is less than 2 per cent of the total sulphur. He obtained positive results in 58 of 59 cases of cancer, and in all of 5 cases of tuberculosis; in these latter the neutral-sulphur in this fraction was from 2.9 to 4.3 per cent of the total sulphur. Saxl<sup>48</sup> says that the protein metabolism in cancer resembles that seen after feeding sulphocyanates to normal persons, there being increased excretion of ammonia, neutral sulphur and oxyproteic acids; the same urinary changes are seen in advanced tuberculosis and are indicative of interference with tissue oxidation. Saxl gives figures on the sulphocyanate excretion in various diseases, finding the highest figures in cancer, generally 100 to 200 mgm. per day, while in tuberculosis the figures were nearly as high, being from 80 to 136 mgm. He does not give enough figures to establish the usual sulphocyanate excretion in tuberculosis as compared with diseases other than cancer.

*Urochrome, urochromogen and the diazo reaction*

As indicated above, in the mixture of unknown substances that constitute the proteic acid fraction of the urine are contained the coloring matters, urochrome and urochromogen. There has been much confusion concerning the relation of these two fractions to one another, and their composition, which does not particularly concern us here. The latest views concerning the nature of chromogen and urochrome are given in detail by Fürth<sup>49</sup> and by Weiss,<sup>50</sup> the substance of their statements being as follows:

<sup>46</sup> Deut. med. Woch., 1912 (38), 53.

<sup>47</sup> Jour. Cancer Res., 1917 (2), 378.

<sup>48</sup> Biochem. Zeit., 1913 (55), 224.

<sup>49</sup> Biochem. Zeit., 1919 (96), 269.

<sup>50</sup> Ibid., 1920 (102), 228.

1. The color of the normal urine depends on the summation of the color of several substances, of which the *urochrome* plays a subordinate rôle. The nature of the urochrome, which is found in normal as well as pathological urines, is entirely unknown.

2. *Urochromogen* is a substance found in appreciable amounts only in pathological urines, especially in conditions associated with tissue destruction or wasting, e.g., cachexia, cancer, tuberculosis, phosphorus poisoning, and other states in which increase in the residual-N fraction of the urine is observed. This urochromogen fraction when oxidized with a weak solution of permanganate gives a color resembling that of urochrome, which Weiss formerly supposed it to be, but it is now doubted that urochrome is merely a more completely oxidized urochromogen. Although a considerable part of the neutral sulphur of the urine comes down in the urochromogen fraction, it is by no means certain that this is a part of the urochromogen itself.

3. The urochromogen fraction contains the substance of the urine which, when treated with Ehrlich's diazo reagent followed by ammonia, gives the typical "diazo reaction." If the urine gives a positive *Ehrlich diazo reaction*, it will also give the permanganate test for urochromogen. The nature of the substance giving the diazo reaction has not yet been determined, but it is probably a phenol or a phenol derivative.

4. The *Penzoldt-Pauly diazo reaction*, which is given by the normal urine even after removal of the coloring matter, when treated with Ehrlich's reagent followed by NaOH solution, is apparently entirely unrelated to the substance which is found in pathological urines and which gives the Ehrlich diazo reaction with ammonia. It probably depends on the presence of a derivative of histidine.

In the present unsettled and developing state of knowledge concerning these substances it does not seem profitable to discuss their possible chemical nature further. They are of interest in the study of tuberculosis because they are apparently indices of tissue katabolism which is abnormal either quantitatively or qualitatively, and because there exist tests that give at least quantitative information concerning their excretion. Apparently all these substances are derived chiefly if not solely from the body tissues.

Pelkan<sup>51</sup> found that in normal subjects a low protein diet reduces the daily urochrome excretion, and a high protein diet increases it. Since in proteins a substance was found giving the color tests for urochrome, he concluded that a large proportion of urinary urochrome must come from the food proteins. However, in starvation the excretion of urochrome continues, and as the highest values for urochromogen are often found in patients who have taken little or no protein food for some time (e.g., tuberculous meningitis, typhoid fever), the endogenous origin of most of the urochromogen, at least, seems probable. Fürth found also that the normal urinary constituent giving the Penzoldt-Pauly diazo reaction is independent of the food intake, and considers it to be of endogenous origin.

We have to deal here, therefore, with either three or four substances found in the proteic acid fraction of urine; two normal constituents of the urine, urochrome and the colorless diazo-reacting body of the Penzoldt-Pauly test; and constituents found in appreciable quantities only as a sign of abnormal tissue breakdown, constituting the urochromogen fraction, which give the permanganate test, and the substance or substances giving the Ehrlich diazo-reaction, which is associated with the urochromogen, but which may be a different substance.

#### *Urochrome in tuberculosis*

Although the urochrome is a constituent of normal urine, the amount seems to be distinctly increased in all diseases where there is much tissue destruction or wasting, and in direct proportion to this excessive tissue catabolism. Weiss<sup>52</sup> gives relative figures, obtained by comparing the color obtained by oxidizing the urine with permanganate solution, with the color of a standard "Echtgelb" solution, in a large number of cases of different diseases. The normal figures usually run from 1200 to 1400 units; in pulmonary tuberculosis his figures for urochrome range in 6 first stage cases from 1248 to 3620 units, averaging 2025; in 2 second stage cases, each gave 2340 units; in 12 third stage cases the range was from 1716 to 5060, average 2769 units.

<sup>51</sup> Jour. Biol. Chem., 1920 (43), 237.

<sup>52</sup> Biochem. Zeit., 1911 (30), 349.

The coloring matter described as urochrome by Dombrowski is probably not a natural constituent of the urine, but a product of oxidation during the chemical manipulation. Hence the figures on the quantity of this fraction of the urine, which Weiss published in some of his earlier work,<sup>53</sup> have no significance, and will not be repeated here.

### *Urochromogen*

The presence of urochromogen is determined by the increased yellow color produced by oxidizing the urine, or the separated proteic acid fraction, with 0.1 per cent potassium permanganate. This reaction is never given by normal urine, and seems to be a distinct evidence of the excretion of incompletely oxidized products of tissue catabolism. The amount excreted is not at all influenced by the diet, and it is solely of endogenous origin. As such conditions as chloroform or phosphorus poisoning are accompanied by increased urochromogen excretion, it seems probable that it is a product of tissue destruction under asphyxial conditions. As a rule, the diazo reaction of Ehrlich is found only in urines which give the permanganate test for urochromogen, and the intensity of the two reactions usually varies with one another, indicating the possibility that they depend on the same or closely associated substances.

As for the urochromogen in tuberculosis, Weiss<sup>54</sup> states that a positive reaction is never given in the first stage of tuberculosis, and that its presence indicates actively progressing lesions. Of course, this is with the assumption that the reaction does not depend on something else than the tuberculosis itself, for it may result from a tuberculin reaction and from chloroform necrosis, in the last case presumably from the tissue destruction that is known to result from chloroform. The reaction usually appears when the previously local tuberculosis begins to cause systemic intoxication and sometimes before this is otherwise clinically manifest. As the disease progresses the strength of the reaction increases, but shortly before death it may disappear, especially if there are

<sup>53</sup> Biochem. Zeit., 1910 (27), 203.

<sup>54</sup> Münch. med. Woch., 1911 (58), 1348.

renal lesions.<sup>55</sup> In early active tuberculosis the reaction may appear and disappear with acute exacerbations and their subsidence. While positive reactions are seen in acute infectious diseases, such as typhoid, here they are not of such bad prognostic significance. Positive reactions in tuberculosis represent a degree of systemic intoxication that occurs only in severe conditions of this disease, but which appear early in acute infections. These general conclusions have been supported by later investigators.<sup>56</sup>

Gottschalk<sup>57</sup> says that repeated, approximately quantitative estimations of the urochromogen reaction, plotting the results in curves, gives valuable information as to prognosis, the curve rising as the disease becomes more active. He agrees with many others that this reaction appears earlier, is more sensitive and more constant than the diazo reaction, which is discussed below. In surgical tuberculosis the urochromogen reaction is considered by Düttmann<sup>58</sup> to be of value in indicating the need of radical intervention. If it fails to disappear after operation this has not been complete or there is an active focus elsewhere. With renal amyloidosis the reaction persists even after radical surgical treatment.

### *The diazo-reaction*

Ehrlich in 1882 reported that if diazo compounds of the aromatic series<sup>59</sup> are added to certain pathological urines, upon subsequent alkalinizing with ammonia a red color is produced. This was found to be especially marked in certain acute infectious diseases, notably typhoid, and also in some cases of febrile tuber-

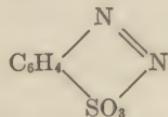
<sup>55</sup> See Stepp, Münch. med. Woch., 1918 (65), 560.

<sup>56</sup> See Guth, Beitr. Klin. Tuberk., 1920 (45), 198.

<sup>57</sup> Beitr. Klin. Tuberk., 1922 (51), 1.

<sup>58</sup> Beitr. klin. Chir., 1921 (123), 454.

<sup>59</sup> The diazo compound in the classical Ehrlich reaction is p-diazobenzene-sulphonic acid,



For a discussion of the chemistry of this reaction see Koessler and Hanke, Jour. of Biol. Chem., 1919 (39), 497.

culosis. As has been stated previously, Weiss found that this depends on the presence of urochromogen, which is not normally present in the urine, but which, under conditions of deficient tissue oxidation with tissue destruction, is excreted in place of the more completely oxidized urochrome. Whether the chromogen itself, or something precipitated along with it, is the reacting substance is not known. Urine may contain many substances besides the chromogen that are capable of giving color reactions with aromatic diazo compounds, including tyrosine, p-oxyphenylpropionic acid, and p-oxyphenylacetic acid, histidine and imidazolaminoacetic acid, as well as other possible compounds derivable from histidine

and containing the imidazol ring,  $\text{N} \begin{array}{c} \text{C} - \text{C} \\ | \\ \text{C} - \text{N} \end{array}$ ; also simpler phenols,

as paracresol. Guth<sup>56</sup> states that orthocresol or metacresol inhibit the reaction with paracresol, and suggests that the urochrome and the urochromogen may be isomers of a compound, the urochrome representing the para, the urochromogen a mixture of the ortho and meta forms, for he found that sometimes careful treatment with permanganate of a urine which gave a positive permanganate but a negative diazo reaction, led to the production of a positive diazo reaction; continued oxidation with permanganate prevents the diazo reaction. Weiss<sup>50</sup> believes that the Ehrlich diazo reaction depends on a phenol derivative, perhaps an alkaption body, closely related to the substance in the urochromogen fraction which gives the permanganate reaction. Hermanns and Sachs<sup>60</sup> consider the reactive substance to be an ethereal sulphate of some oxidation product of tyrosine and that more than one substance may occur in urine giving the Ehrlich diazo reaction. In a case of carcinoma of the liver they found a derivative of tryptophan, most probably hydroxyindoleacetic acid.

There is an enormous literature on the diazo reaction in tuberculosis, the earlier portion of which is reviewed by Clemens.<sup>61</sup> There is general agreement that it is of little diagnostic value, since it is not a specific reaction for tuberculosis but for exaggerated

<sup>56</sup> Zeit. physiol. Chem., 1921 (114), 79 and 88.

<sup>61</sup> Ott's Chem. Path. d. Tuberculose, p. 212.

and perhaps qualitatively abnormal tissue disintegration, and especially since in tuberculosis it appears only in advanced and active cases in which more reliable evidences of tuberculosis are obtainable. There is equally good agreement that when persistently present the diazo reaction indicates a degree of tissue disintegration which, if caused by tuberculosis, occurs almost only in cases so far advanced and so active that a favorable prognosis cannot be given; but even here exceptions have often been observed. It is not the location of the tuberculosis lesion, nor its anatomical features, that determine the reaction, but merely the amount of toxicogenic destruction of protein. This is especially well shown by the fact that patients who give a negative diazo reaction may show a strong positive test during the febrile period of a tuberculin reaction. If both the urochromogen (permanganate) test and the diazo reaction are followed in the same patient it will usually be found that the former appears much earlier than the diazo test, although sometimes they appear together (Guth) and their clinical significance is much the same.

Weiss<sup>54</sup> says that in severe tuberculosis, as well as in all other conditions that are accompanied by a positive diazo reaction, the destruction of the tissue proteins is not only increased in amount, but also in a qualitatively different manner from the normal catabolism. Conditions that are accompanied by a positive diazo reaction can be considered as toxic, in the broadest sense of the word. Bacterial poisons most usually are responsible, and their influence in severe cases seems to be such that the oxidation processes, which normally lead to the formation of urochrome, stop short at the less oxidized antecedent urochromogen.

Bosch<sup>62</sup> found that the diazo reaction was never obtained in the urine in tuberculosis without a positive permanganate (urochromogen) reaction, but the latter was obtained more than twice as often as the diazo reaction. Occasionally a case of tuberculosis is observed in which a positive urochromogen reaction is obtained only transitorily; these sporadic reactions do not have the unfavorable prognostic significance of the persistent reactions.

<sup>52</sup> Deut. med. Woch., 1916 (42), 17.

Wahlberg<sup>63</sup> found the diazo reaction positive in but 15.3 per cent of 294 cases of pulmonary tuberculosis while the Weiss urochromogen reaction was positive in 50 per cent of these cases.

Bonnert<sup>64</sup> has compared these two reactions in 1000 cases of pulmonary tuberculosis, finding both negative in 850 cases, both positive in 108 cases, an agreement in 95.8 per cent of all cases. In 20 of 36 cases of amyloidosis both reactions were positive at the same time; in 13 cases the urochromogen reaction appeared one month before the diazo reaction, in 3 cases they were both constantly negative. The agreement is generally so good that Bonnert considers that the Weiss urochromogen reaction is a useful substitute for the diazo reaction, of great value especially for the practitioner on account of the simplicity and cheapness.<sup>65</sup> The urine in 116 cases of pulmonary tuberculosis was analyzed at frequent intervals by Guth,<sup>56</sup> who found that generally the Weiss reaction precedes the diazo reaction in appearance, often by a considerable time; occasionally they appear simultaneously, but rarely is the diazo reaction first. A persisting positive reaction was usually of bad prognostic significance.

Williams<sup>66</sup> has reported that the diazo reaction is rarely given by negroes with degrees of tuberculosis that usually produce this reaction in white subjects, and has kindly furnished me with a report on 100 additional unpublished cases with similar results. Other southern physicians, however, have not found this difference between black and white patients. F. B. Johnson of Charleston, South Carolina, has given me the following figures: In 52 cases of tuberculosis, the urine in 30 per cent was positive to the diazo

<sup>63</sup> Finska Läkar. Handlingar, 1921 (63), 360.

<sup>64</sup> Tubercle, 1921 (2), 537.

<sup>65</sup> The test is performed as follows: In a test tube about 5 cc. of urine are diluted with water to treble the original volume. Well mixed, the contents are then divided equally in two test tubes of the same diameter. To one of them one drop of a 1:1000 solution of potassium permanganate is added. The other tube serves as a control. If the reaction is positive, a canary yellow color of varying intensity, which keeps stable for hours, is obtained after shaking. If the reaction is negative, no change of color appears, or only a yellow or brown color of short duration (from half to one minute). Thus the test ought not to be read off until after one minute.

<sup>66</sup> Med. Record, 1907 (71), 480.

test, 40 per cent to the Russo test, and 62 per cent to the Weiss urochromogen test. Of these, 31 were negroes, with 25.8 per cent positive to the diazo test, 30 per cent to the Russo test, and 61 per cent positive to the Weiss test. Hence there is in this series little difference between blacks and whites. Other physicians have informed me of similar results. As negroes give strong diazo reactions in typhoid and malaria it is improbable that they exhibit any essential difference from white men in their chemistry in tuberculosis.

#### *The Penzoldt-Pauly diazo reaction*

As stated previously, this is given by the normal urine, and as the reacting fraction can be separated from the coloring matter of the urine, it is quite distinct from the Ehrlich diazo reaction in significance. Fürth and Weiss agree that it probably depends on the presence of some derivative of histidine. Only Fürth has considered this reaction in relation to tuberculosis. He says that the "diazo value" of normal urine (which he calculated as histidine monochlorid)<sup>67</sup> amounts to 0.03 to 0.07 (average 0.044) in 100 cc. urine, or 0.3 to 0.6 in twenty-four hours. The "diazo quotient," which is the per cent of the total nitrogen that this diazo fraction constitutes, is from 0.38 to 0.78, (average 0.6). Quite the normal figures were obtained in early tuberculosis with good nourishment, whereas in advanced tuberculosis with cachexia the diazo quotient rose to 0.8 to 1.3. In chronic protein deficiency, with but 6.8 grams N daily excretion, the absolute diazo value is very low but the quotient is high (average 0.83). This fraction of the urine, therefore, bears the same evidence of tissue loss in tuberculosis as the other members of the proteic acid fraction.

*Russo's methylene blue reaction*, as well as other tests which depend on the production of a green color when a blue dye is added to the urine, all rest on the fact that yellow urine plus blue dye make a green color. They are merely evidence of the degree of yellowness of the urine. As in advanced tuberculosis the amount of urochrome excretion may be increased,

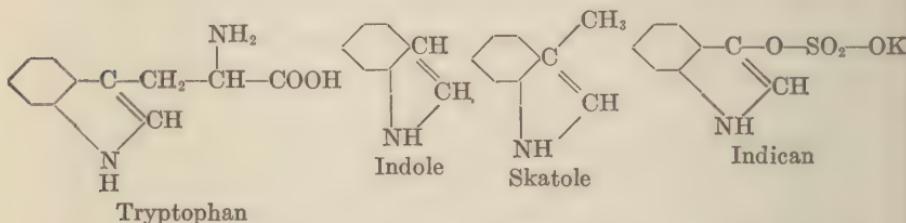
<sup>67</sup> The method used by Fürth did not exclude the presence in his solutions of other substances than histidine giving the Penzoldt-Pauly reaction, and hence his figures are much too high for the histidine or its derivatives present in the urine.

positive tests may be given with these blue dyes, but they have little or no significance since bile pigments will have the same effect and also the normal increase of the color in concentrated urines will cause positive reactions.<sup>68</sup>

#### OTHER AROMATIC COMPOUNDS

##### *Indole and indican*

There can be no question that the greater part of the indican excreted in the urine comes from indole formed in the intestine by bacterial decomposition of proteins, the tryptophan of the protein being its direct source. During long fasts the urine has been found free from indican, which indicates that probably none is of endogenous origin; also if gelatin, which contains no tryptophan, is substituted for the proteins of the diet there is no excretion of indican (Underhill). The relation of tryptophan to indole, skatole and indican is shown by their structural formulae.



Tryptophan

If indican is found increased in the urine in tuberculosis, therefore, this ordinarily will depend on intestinal stasis, although it is possible that unimportant additions might come through the bacterial decomposition of gangrenous foci in the lung, since occasionally indole has been found in the sputum from this source. Normally the urine should contain little or no indican, but usually the urine of a healthy adult contains about 10 to 20 mgm. per day.<sup>69</sup> Most of the early observations on indicanuria are of little value because of faulty methods of determination. There is no reliable evidence that pulmonary tuberculosis itself causes any increased indicanuria, although possibly asthenic

<sup>68</sup> See Skutetzky and Klaften, Wien. klin. Woch., 1918 (31), 1016. Review of literature by Boit, Beitr. Klin. Tuberk., 1918 (38), 154.

<sup>69</sup> A review is given by Houghton, Amer. Jour. Med. Sci., 1908 (135), 567.

conditions may lead to intestinal stasis and putrefaction with resulting increase in the indican. Usually, however, noteworthy indicanuria in tuberculosis means intestinal ulceration with stasis or increased putrefaction,<sup>70</sup> and Hoppe-Seyler<sup>71</sup> found enough in the urine of a patient with intestinal tuberculosis to isolate and determine positively as indican together with a little of the glycuronic acid compound of indole.

The blood serum normally contains from about 0.025 to 0.075 mgm. of indican per 100 cc., the amounts being raised in the blood when there is indicanuria, and also when there is impaired renal elimination. Haas<sup>72</sup> found normal blood figures in a few cases of pulmonary tuberculosis and high figures in 3 cases of suspected peritoneal tuberculosis.

Other aromatic compounds are formed in the intestine and in gangrenous areas under the same conditions as indole, and hence we may expect to find them increased under the same conditions as the urinary indican, but we have little evidence concerning their excretion in tuberculosis. It is to be expected that they will be found increased in intestinal obstruction, whether from ulcers or from tuberculous peritonitis. The chief points of importance concerning the best known of these aromatic compounds are given below.

*Skatole* seems to accompany indole in small amounts, but apparently in no constant quantitative relation. Herter<sup>73</sup> states that skatole is formed under entirely different conditions from indole and that *B. coli* does not produce skatole. It is not always present in the contents of the large intestines of healthy persons, and seems to be formed later than indole. We find no reports concerning skatole in the urine in tuberculosis, but Clemens<sup>74</sup>

<sup>70</sup> An exception to the generally accepted statement concerning the origin of indican is made by Moraczewski (*Zeit. klin. Med.*, 1914 (79), 248), who reports that when indican is increased there is also a parallel increase in uric acid, acetone and oxalic acid, which he interprets as indicating that all are of endogenous origin from tissue wasting. Among his cases with high indican and uric acid were some of advanced pulmonary tuberculosis.

<sup>71</sup> *Deut. med. Woch.*, 1916 (42), 1213.

<sup>72</sup> *Deut. Arch. klin. Med.*, 1916 (119), 117.

<sup>73</sup> *Jour. Biol. Chem.*, 1908 (4), 101; general discussion.

<sup>74</sup> Ott's *Chem. Path. Tuberk.*, p. 208.

considers that the darkening of the urine on standing, often reported in tuberculosis, depends on skatole or related substances. Sometimes, however, this darkening may depend on a true *alkaptonuria*,<sup>75</sup> a few such cases having been described in tuberculosis.<sup>76</sup>

*Indole-acetic acid* appears in the normal urine in extremely minute quantities, and is increased in the same conditions as skatole. It is the mother substance of *urorosein*, and can be found in the intestines of patients who show this substance in the urine (Herter).<sup>77</sup> Ross<sup>78</sup> found indole-acetic acid in the urine in 21 per cent of normal persons, and in 48 per cent of dementia precox cases, and obtained evidence in favor of an endogenous origin in 2 cases studied especially to determine this point. In 26 insane persons with tuberculosis he found 10, or 38.5 per cent, to give a positive test, so that the figures are not significantly altered by the tuberculosis. Blumenthal<sup>79</sup> obtained a positive Salkowski color test for "*skatole carbonic acid*," really indole-acetic acid, in the urine in cases of intestinal tuberculosis, advanced pulmonary tuberculosis, as well as in gastro-intestinal cancer, pneumonia, pyemia and peritonitis.

*Phenylacetic acid*<sup>80</sup> is formed from phenylalanine on putrefaction, as also are *phenylpropionic* and *benzoic acids*.

*Benzoic acid* combines with glycine and is excreted as *hippuric acid*. The amount of hippuric acid in the urine depends chiefly on the amount of benzoic acid in the food, and the amount formed in the intestines by putrefaction. Lewin<sup>81</sup> found normal amounts of hippuric acid in the urine in afebrile tuberculosis, increased amounts sometimes but not always in febrile cases, the patients being on a fixed diet. He found that there is usually an increase in all febrile conditions, suggesting the possibility that some of it at least is of endogenous origin. *Phenylpropionic acid* is excreted as p-hydroxyhippuric acid.

<sup>75</sup> See Wells, Chemical Pathology, 4th Ed., p. 586.

<sup>76</sup> Moraczewski, Cent. inn. Med., 1896 (17), 177; Gebhardt, Virch. Arch., 1913 (213), 312.

<sup>77</sup> Jour. Biol. Chem., 1908 (4), 253.

<sup>78</sup> Arch. Int. Med., 1913 (12), 112 and 231.

<sup>79</sup> Charité Annalen, 1902, quoted by Clemens in Ott, p. 206.

<sup>80</sup> See Sherwin and Kennard, Jour. Biol. Chem., 1919 (40), 259.

<sup>81</sup> Zeit. klin. Med., 1901 (42), 371.

*Phenols*<sup>82</sup> appear in the urine normally in very minute quantities—from 0.005 to 0.07 gram per day, according to various observers. These figures are undoubtedly too low, for Folin and Denis<sup>83</sup> found the total excretion of phenols to be from 0.2 to 0.4 gram per day, the amount varying with the protein intake. They seem to come chiefly, if not entirely, from tyrosine.<sup>84</sup> Much more is undoubtedly formed in the intestines, for but a small fraction of phenol given by mouth (2 to 3 per cent according to Munk) appears in the urine as a sulphuric-acid compound; part of the rest is oxidized to hydrochinon and pyrocatechin,  $C_6H_4(OH)_2$  and eliminated as ethereal sulphates. These sulphates, although distinctly toxic, are much less so than the phenol itself (Metchnikoff).<sup>85</sup> Contrary to prevailing ideas, Folin and Denis found the greater part of the phenols to be excreted uncombined. The largest quantities are found in the same conditions as indican except, of course, in "carbolic-acid" poisoning. A small amount of urinary phenol may be of endogenous origin. We have no recent observations on urinary phenol excretion in tuberculosis but Folin and Denis state that in a great number of examinations they found no evidence of a specifically abnormal production of phenols in connection with any one disease. Hence the statements in the older literature, as quoted by Clemens, are of little or no value, although Blumenthal was probably correct in his finding that in pulmonary tuberculosis there was no increase in the phenols, but in intestinal tuberculosis they were increased as well as in conditions with putrid infections.

Blood contains small amounts of free phenols, about one-third being polyphenols. In a series of pathological cases Theis and Benedict<sup>86</sup> found from 1.87 to 7.96 mgm. per 100 cc. blood, somewhat higher figures being found in sarcoma and hernia cases than in other diseases. Unfortunately they give no figures in pulmonary tuberculosis.

*Cresol* (chiefly paracresol), *para-oxyphenyl acetic acid*, and *para-oxyphenyl propionic acid* appear under similar conditions, except

<sup>82</sup> Literature given by Dubin, Jour. Biol. Chem., 1916 (26), 69.

<sup>83</sup> Jour. Biol. Chem., 1915 (22), 309.

<sup>84</sup> Tsudji, Jour. Biol. Chem., 1919 (38), 13.

<sup>85</sup> Ann. Inst. Pasteur, 1910 (24), 755.

<sup>86</sup> Jour. Biol. Chem., 1918 (36), 99.

that the two oxy-acids are possibly also formed within the body through cellular metabolism, as they have been found present in the urine independent of intestinal putrefaction. Paracresol is quantitatively the most important of the urinary phenols.

*Rosenbach's reaction* (burgundy-red color on boiling urine with nitric acid, followed by neutralization) occurs in urine that is rich in the above aromatic substances, and was formerly studied to some extent. Ewald<sup>87</sup> found only mild reactions in phthisis, especially when associated with diarrhea, the results generally paralleling the indican reaction in intensity. Abraham<sup>88</sup> found more or less of a reaction in 12 cases of pulmonary tuberculosis with fever, and no reaction in 16 cases without fever.

#### UROBILIN<sup>89</sup>

This pigment is probably formed chiefly, if not solely, from bile pigments by the action of reducing bacteria in the intestine. It is excreted in the urine only as its chromogen, urobilinogen, but in the feces both urobilin and urobilinogen may be found; when exposed to air the chromogen oxidizes quickly to urobilin. In the liver the urobilin is largely worked over to form new hemoglobin, and hence the functional capacity of the liver is indicated by the completeness with which it utilizes the urobilin, except in cases of excessive formation of urobilinogen as a result of hemolysis. The amount of urobilinogen in the urine will be found increased, therefore, in hemolytic icterus, and decreased in obstructive icterus. Exceptionally, urobilinogen may be formed from blood disintegrated in bloody effusions without evident participation of the liver, e.g., urobilinogenuria with hemorrhagic pleurisy. With a normal liver urobilinogenuria is found only when there is excessive hemolysis, otherwise urobilinogenuria occurs only with an injury to the liver parenchyma (Hildebrandt). In general, the amount in the urine is an index of the amount of blood destruction.<sup>90</sup> Normally there is a very small amount of urobilinogen

<sup>87</sup> Berl. klin. Woch., 1889 (26), 953.

<sup>88</sup> Ibid., 1890 (27), 385.

<sup>89</sup> Bibliography and review by Meyer-Betz, Ergeb. inn. Med., 1913 (12), 734; Wilbur and Addis, Arch. Int. Med., 1914 (13), 235.

<sup>90</sup> Dubin, Jour. Exp. Med., 1918 (28), 313.

and related substances in the urine, which disappears when there is no bile in the intestine. The amount of urobilin and urobilinogen excreted in the feces, seems to vary directly with the amount of hemolysis,<sup>91</sup> and the same is true for the duodenal contents.<sup>92</sup> The evidence of abnormal hemolysis is said to occur first in the stools, then in the duodenal contents, and lastly in the urine.

The extensive study of this substance in various diseases by Wilbur and Addis<sup>89</sup> makes no mention of tuberculosis except to report 1 case of tuberculous peritonitis with a large amount of blood in the peritoneal cavity, with much urobilin in the urine. A similar finding is to be expected in other tuberculous conditions with internal hemorrhages or bloody exudates, and with hepatic lesions in tuberculosis.

Meyer-Betz<sup>89</sup> reports that although 83 per cent of a series of cases of pneumonia gave a positive test for urobilinogen in the urine, it was missing in early stages of pulmonary tuberculosis, but strong reactions are often found in advanced stages, this being considered a bad prognostic sign, since it indicates either excessive hemolysis or considerable hepatic damage or both. Although slight or no reactions were obtained in a few cases of miliary tuberculosis, strong reactions were obtained in some cases of generalized lymphatic tuberculosis and in tuberculous peritonitis, and it may appear during a tuberculin reaction. Internal hemorrhages or abscess formation increase the reaction, while diarrhoea may reduce it.

Considering the above variations in tuberculosis, and the varied conditions in which positive reactions are obtained, it is evident that in this disease the diagnostic value of urobilinogen determination is little or nothing.

#### *Para-dimethylaminobenzaldehyde reaction*

The *Para-dimethylaminobenzaldehyde reaction* of Ehrlich was at first supposed to depend upon the presence in the urine of a carbohydrate derived from protein disintegration.

<sup>91</sup> Robertson, Arch. Int. Med., 1915 (15), 1072; McCrudden, Bost. Med. Surg. Jour., 1917 (177), 907.

<sup>92</sup> Giffin, Sanford and Szlepka, Amer. Jour. Med. Sci., 1918 (155), 562.

Neubauer showed that this reaction is given by urobilinogen and it is now used as a colorimetric method for the quantitative estimation of this substance. When the urobilinogen is oxidized to urobilin the aldehyde reaction is diminished. Neubauer stated further that the reaction is given with feces because of the presence of indole,<sup>93</sup> skatole and urobilinogen. In general, the Ehrlich aldehyde reaction depends on the presence of pyrrol derivatives. The Ehrlich diazo reagent test (see p. 333) gives an orange-yellow with urobilinogen, in the absence of urochromogen.

The aldehyde reagent gives with normal urines as a rule only a faint red color, but in some pathological states a darker, cherry red color is observed. Ehrlich obtained such positive reactions in typhoid, phthisis and chronic enteritis, and Clemens<sup>94</sup> obtained 9 positive reactions among 26 cases of pulmonary tuberculosis in different stages. Generally the positive results are obtained in more advanced cases of tuberculosis, but it does not always correspond to the Ehrlich diazo reaction although they often are present in the same specimens. Koziczkowsky<sup>95</sup> obtained positive reactions in 5 premortal cases of pulmonary tuberculosis and occasional reactions in 5 other cases during periods of exacerbation of the disease. Berkowitz<sup>96</sup> obtained positive results in such unrelated and inexplicable conditions that he repudiates any possible diagnostic value for the aldehyde reaction.

A study of the aldehyde and diazo reactions in 50 cases of pulmonary tuberculosis in moderate to severe conditions for periods of four to twelve weeks by Kornrumpf<sup>97</sup> showed that only persistent reactions are of significance, and even these did not correspond accurately to the stage or progress of the disease. As a rule the aldehyde reaction was found more frequently and usually earlier than the diazo test, there having been 10 cases with the aldehyde reaction only, one with diazo test only, 18 with both and 20 with neither. He agrees with Berkowitz that both reactions are too inconstant to be even of prognostic value.

<sup>93</sup> The aldehyde reaction is commonly used as a test for indole in bacteriology (see Norton and Sawyer, *Jour. of Bact.*, 1921 (6), 471).

<sup>94</sup> Deut. Arch. klin. Med., 1901 (71), 168.

<sup>95</sup> Berl. klin. Woch., 1902 (39), 1029.

<sup>96</sup> Med. Record, 1914 (86), 1087.

<sup>97</sup> Med. Klinik, 1921 (17), 324.

Hári<sup>98</sup> has reported that, as with the diazo reaction, there are two different substances to be found in urine giving this aldehyde reaction. One, that described by Ehrlich, is found in pathological urines and gives the reaction in the cold. The other, which is present in normal urine, gives the reaction only when the urine is heated, is not increased in cases in which the other aldehyde-reacting substance is present, and Hári believes that these two substances are entirely different from one another.

#### ORGANIC ACIDS

##### *Acetonuria*

Acetonuria, this term covering the entire group of "acetone-bodies," is commonly looked upon as an evidence of deficient tissue oxidation, but we have little evidence concerning its occurrence in tuberculosis. Without giving authority, Winternitz<sup>99</sup> says that in severe phthisis is found not only increased acetone excretion, but also aceto-acetic acid in the stage of advanced undernourishment, in proportion to the nutrition. v. Jaksch in 1884 found that in febrile tuberculosis only is there an increased acetonuria. The appearance of these acetone bodies depends on disintegration of body tissue, both protein and fat, usually when there is a deficiency in available carbohydrate. Of course much larger quantities are found in tuberculosis associated with diabetes, but here the latter is to be considered responsible.

More exact information we have been unable to find, beyond the mention by Garland<sup>100</sup> of acetonuria in 5 cases of miliary tuberculosis in children. Since acetonuria is a regular accompaniment of starvation and cachexia, it is undoubtedly present in advanced pulmonary tuberculosis, but we lack any exact studies by modern methods.

The presence of lactic acid in the urine is more direct evidence of decreased oxidation,<sup>101</sup> but we can find no report on its presence

<sup>98</sup> Biochem. Zeit., 1921 (117), 41.

<sup>99</sup> Brauer, Schroeder and Blumenfeld's "Handbuch der Tuberkulose," 1914 (2), 74.

<sup>100</sup> Arch. Pediatrics, 1919 (36), 468.

<sup>101</sup> See Wells, Chemical Pathology, 4th Ed., p. 564.

or absence in tuberculosis beyond a few casual observations made so long ago that the results cannot now be accepted.

Diacetic acid was found in the urine of cases of rapid miliary tuberculosis, in 6 cases of tuberculous meningitis and in but 2 of 35 cases of pulmonary tuberculosis in children, by Schrack.<sup>102</sup> Montanari<sup>103</sup> has made similar observations in seven cases of tuberculous meningitis, finding also sugar and acetone.

Ammonia excretion is an index of excretion of acetone bodies, although other acids are also more or less neutralized by ammonia. Consequently in febrile conditions generally there is an increased excretion of ammonia. Leube<sup>104</sup> found three times the normal ammonia excretion in a febrile phthisis case as compared with a normal person on the same diet.

#### *Oxalic acid*

The older authors believed that there might be an increased excretion of oxalic acid in tuberculosis because of decreased oxidation, but we have at present no reason to believe that decreased pulmonary ventilation increases the excretion of oxalic acid. A. Mayer<sup>105</sup> reports that in febrile tuberculosis there is a constant increase in the urinary oxalic acid. He found in normal urine an average of 0.015 gram per day, while in tuberculosis he found from 0.05 to 0.08 gram per day. Usually the urine in tuberculosis is so acid that precipitation of calcium oxalate is not observed. He did not find that this increase depended upon dyspnea, but as it was observed that staphylococci and streptococci form oxalic acid when growing on blood, probably from sugar, he thought that the increased amount in tuberculosis is derived from suppuration. Another possible source is in increased nucleic acid destruction, since oxalic acid may be formed from the purine ring.

#### URINARY FERMENTS

The excretion of ferment in the urine is inconstant and trivial at best, and hence variations in disease have little or no sig-

<sup>102</sup> Jahrb. f. Kinderheilk., 1889 (29), 411.

<sup>103</sup> Rivista clin. Ped., 1921 (19), 40.

<sup>104</sup> Quoted by Clemens as Virch. Arch., (53), 209, but this reference is incorrect.

<sup>105</sup> Deut. Arch. klin. Med., 1907 (90), 425.

nificance.<sup>106</sup> There are a few references to observations on urinary enzymes in tuberculosis in the literature, which are cited below.

White and Zeedick<sup>107</sup> report that in normal urine from patients with recovering, afebrile tuberculosis there is little if any *lipase*, but in advanced cases with fever and oncoming dissolution there is a rise in lipase (splitting ethyl-butyrate). This lipase increase precedes the fever, and declines with defervescence. In cachexia there may be a diminution in the urinary lipase.<sup>108</sup>

*Catalase* content varies directly with the quantity of cells or cellular disintegration fragments in the urine, leucocytes being particularly rich in this enzyme.<sup>109</sup> Hence it will be abundant in renal tuberculosis with suppuration, but scanty otherwise and not particularly affected by extrarenal conditions. With disintegration of the renal epithelium in toxic states there may be a slight rise in urinary catalase.

Since the *diastase* present in the urine seems to come chiefly from the pancreas and salivary glands,<sup>110</sup> neither of which tissues is likely to be affected in tuberculosis, its determination in the urine in this disease has no significance except that marked renal injury decreases the amount, and hence in tuberculosis of the kidney the diastase activity of the urine from the diseased side is decreased.<sup>111</sup>

Stadelmann<sup>112</sup> has reported that in a case of febrile phthisis with hemoptysis he found an increased *pepsin* activity in the urine. Hedin<sup>113</sup> has found an erepsin always present in both normal and pathological urine. In pneumonia the urine contains an enzyme attacking casein in alkaline solution, and all forms of proteolytic enzymes are increased in albuminous urines, but he makes no reference to the urine in tuberculosis.

<sup>106</sup> See Jacoby in Neuberg's "Der Harn," 1911 (1), 845.

<sup>107</sup> Trans. Assoc. Amer. Phys., 1916 (31), 111.

<sup>108</sup> Pribram and Loewy, Zeit. physiol. Chem., 1912 (76), 489.

<sup>109</sup> See Norgaard, Jour. Biol. Chem., 1919 (38), 501.

<sup>110</sup> For literature see McClure and Pratt, Arch. Int. Med., 1917 (19), 568.

<sup>111</sup> See Corbett, Quart. Jour. Med., 1913 (6), 351; Block, Zeit. klin. Med., 1922 (93), 381.

<sup>112</sup> Zeit. f. Biol., 1889 (25), 208.

<sup>113</sup> Zeit. physiol. Chem., 1920 (112), 252.



## **SECTION III**

### **THE CHEMOTHERAPY OF TUBERCULOSIS**

BY  
LYDIA M. DEWITT



## CHAPTER XIII

### THE CHEMICAL BASIS OF THE THERAPEUTICS OF TUBERCULOSIS

In Ott's *Chemische Pathologie der Tuberkulose*, Schrötter, treating of climatic therapy of tuberculosis, emphasizes the necessity of studying climatology from the chemico-physical point of view. He urges that we need to consider the effect on blood corpuscles and on blood plasma, on body temperature, on the metabolism of the patient and other vital conditions which may be favorably or unfavorably influenced. How much more important and more necessary is this study as we consider the chemical treatment of the disease. We should know something of the chemical, physical and physiological effect of the disease itself. Of these things, too little is known. The anatomical changes of tuberculosis have long been carefully studied from a morphological standpoint, but such changes must be associated with chemical changes—changes in the tubercle and in the tissue surrounding it, changes in the blood, lymph, and body fluids; changes in the tissues and organs, changes in the secretions and excretions, all changes which may be grouped under the term altered metabolism. All that is known of these changes, of the chemistry of the tubercle bacillus, of tuberculin, of the tubercle, and of the metabolism of tuberculosis has been discussed in Sections I and II.

They need only to be mentioned here as they are related to the problems of chemotherapy. If we wish to destroy the tubercle bacilli in the body, we must consider, not only what chemicals can kill the bacilli in the test tube but also which ones can penetrate the tubercle and pass through the wall of the phagocyte in which it is so often entrenched. We must consider what changes the chemical may bring about in the tissues, in the blood and in the lymph, and what chemical changes may be caused in the compound itself by these tissues. Many things are being discovered as to the nutritional needs of the tubercle bacillus and the metabolic changes which it brings about on the media in the test tube. These things have been discussed in Section I. It is a simple

matter to determine what effect certain drugs may have on the growth of these organisms in the test tube and also what effect the growing organism has on the drugs. We know that the bacilli require oxygen, glycerol and certain nitrogenous compounds, which may be very simple. We may easily, in the test tube, change these conditions of growth or add certain toxic substances which shall either check growth or destroy the organisms. In the test tube, then, we have been able easily to show either a bactericidal or a bacteriostatic action of many drugs.

In the animal body, however, the conditions, as was stated earlier, are quite different. We know too little of the chemistry of the normal tissues, we know less of the chemistry of tissues after the tubercle bacilli begin to grow, we know still less of the chemical changes in these tissues, either normal or tuberculous, caused by different drugs and it is not easy to study them during life. The drugs which have been used in the treatment of tuberculosis are innumerable. Relatively few, however, have been subjected to scientific investigation to determine a reason for their use. Some one reports that a few tuberculous patients improved under a certain drug treatment and at once publishes a report, highly recommending that drug for the treatment of tuberculosis. The number of so-called consumption cures is almost infinite. If, however, we would develop any more rational basis for treatment, we must know what a drug does in the normal body, what are its toxicology and pharmacology. Then we must know a reason for believing that it must act beneficially in a certain manner on the tubercle. This action may be direct—killing the bacilli (bactericidal), or checking their growth (bacteriostatic), or neutralizing their products.

In any case, the drug must be able to penetrate the tubercle, the cell if the bacilli are enclosed within cells, and the tubercle bacillus, which is apparently one of the most difficult of organisms to penetrate. The action of the drug may be indirect, increasing or diminishing phagocytosis or increasing the power of the phagocytes to destroy the bacteria which they have taken up. This might be accomplished by changing the reaction of these cells, making them more acid or more alkaline. As tubercles are usually surrounded by lymphocytes and as the lymphocytes have been said by

Bergel<sup>1</sup> and others to have a lipolytic power, a drug which would increase the number of lymphocytes or increase their lipolytic power might be imagined to influence tuberculosis favorably by breaking down the fatty substances of the tubercle bacilli and thus exposing them to readier destruction by hostile drugs or other changes in their environment.

Certain drugs may act on the surrounding tissue cells (*dynamogenic* action), causing them to proliferate more actively and circumscribe the tubercle, shutting the bacilli off from their oxygen and food supply and allowing their own acid and waste products to check their growth and eventually to destroy them. Among such dynamogenic drugs, Weissmayr<sup>2</sup> lists creosote, guaiacol and camphor. Some drugs may have a chemotactic action and excite an inflammation around tuberculous areas. Weissmayr lists Balsam of Peru, cinnamic acid, cantharidin, carbonic acid, camphor and terpin among the drugs showing such action. Other substances may possibly mineralize the tissue about the tubercle bacilli, making the surrounding tissue so dense that the organisms cannot easily escape and invade the surrounding tissue, and checking their multiplication by preventing the ingress of oxygen and food. Among mineralizing drugs, Weissmayr mentions phosphorus, lecithin, nucleinic acid, and silicic acid. Other drugs, notably certain gold compounds, excite a hyperemia of the tissue surrounding the tubercles and cause a reaction resembling that of tuberculin, the results being beneficial or otherwise, depending on the degree of the reaction. Whatever the mode of action of a drug, the tubercle must be permeated by it, and this question of permeability is one of the most fundamental questions of chemotherapy.

#### THE PERMEABILITY OF TUBERCLES FOR SOLUBLE SUBSTANCES

The conspicuous readiness with which tubercles undergo calcification indicates that substances brought in the plasma do enter even the old, encapsulated tubercle. This fact is of great importance in any consideration of chemotherapeutic treatment of tuber-

<sup>1</sup> Münch. med. Woch., 1909 (56), (64); 1910 (57), 683; Berl. klin. Woch., 1921 (58), 995.

<sup>2</sup> Ott's Chemische Path. der Tuberculose, p. 436.

cles, and has been the object of studies by Loeb and Michaud<sup>3</sup> and later of numerous studies in this laboratory. Loeb and Michaud, as well as Wells and Hedenburg,<sup>4</sup> found that injected iodide of potassium enters freely into tubercles (for details see p. 410). The latter observers state that the proportion of injected iodin in tubercles is usually greater than that in any other tissues of the same animal except the kidneys, where it is being excreted, and it is greater in the caseous contents than in the cellular peripheries of the tubercles. Tuberculous eyes usually contain much more iodin than their normal mates. This property is believed by them to depend on no specific character or affinity of the tubercle itself, for other necrotic tissues also take up more iodin than normal tissues.

The explanation offered is that normal cells are not perfectly permeable to iodides (except perhaps kidney cells) and lose this impermeability or semi-permeability when killed or injured, thus becoming entirely permeable for crystalloids present in the surrounding fluids. As the iodin content of the blood increases and decreases with absorption and elimination, so the iodin varies in the necrotic area, whether tuberculous or otherwise, indicating an absence of any chemical or physical binding of the iodin in such areas. A simple, inert colloid (agar), implanted in the tissues, behaves in quite the same way.

Egg albumen injected into tuberculous pigs was found, by means of the anaphylaxis reaction, to penetrate the avascular tubercles but little if at all, even when present in the blood in large amounts. This agrees with the hypothesis that the passage of iodides from the blood into the tubercles is a purely physical matter, the crystalloidal iodin compounds diffusing through the inert colloidal solution of a necrotic area practically unimpeded, while the colloidal egg albumen, according to the law of colloidal diffusion, is practically unable to diffuse through such a colloidal solution.

No evidence could be found of any tendency for iodin compounds of whatever nature to accumulate in tubercles or other necrotic areas, or to persist in such areas when disappearing from the normal tissues and the blood.

Exudates contain approximately the same proportion of iodin as the blood of the same animals, and hence any area with inflam-

<sup>3</sup> Biochem. Zeit., 1907 (3), 301.

<sup>4</sup> Jour. Infect. Dis., 1912 (11), 349.

matory edema and hyperemia will commonly show more iodin than normal tissues, although not usually more than the blood. No evidence was found of any specific entrance or fixation of iodin in inflammatory exudates. The iodin is distributed about alike in the fluid and solid portions of the exudate, indicating simple diffusion. Of normal tissues only the kidney seems to contain approximately as much iodin as the blood of the same animal. The main features of this work, at least the tendency of iodin to accumulate, not only in tuberculous tissues but also in other necrotizing areas (cancer, syphilis, abscesses) and exudates, have been corroborated by Fujisawa,<sup>5</sup> who seems to have been entirely ignorant of the American work. He attempted to determine the form in which the iodin is bound in these lesions after injection of KI, and found evidence that in tubercles, cancer and syphilis the iodin is united with the lipins, in abscesses and exudates it is found only as inorganic salts, and in no case is it united with the protein.

Lewis and Krauss<sup>6</sup> make the surprising statement that tuberculous tissues of rabbits and guinea pigs frequently contain considerable amounts of iodin even when the animals have received no injections of iodin compounds, this being especially true of the cornea. Sometimes they found iodin demonstrable in normal corneas. It was not usually present in other parts of the eye, whether tuberculous or not, even when the animals had been injected with iodin compounds. As their figures show great variations, and as relatively enormous amounts of iodine are reported in the corneas of untreated rabbits (5.5 and 2.67 mgm. iodin per gram of dried tissue) while the maximum in treated animals was much lower (1.08 to 1.81 mgm.) and the maximum for the thyroid was but 1.565 mgm. and in many thyroids none or bare traces of iodin was found, they cannot be accepted until corroborated by other workers with other methods.

Corper<sup>7</sup> found that fat dyes fed to tuberculous animals never appear in visible amounts in the fatty droplets within tubercles, no matter how thoroughly saturated with the dye the depot fats of the animal may be. As the dyes also fail to appear in the fat

<sup>5</sup> Mitt. med. Fak. Univ. Tokyo, 1918 (19), 445.

<sup>6</sup> Jour. Biol. Chem., 1914 (18), 313; 1915 (22), 159.

<sup>7</sup> Jour. Infect. Dis., 1912 (11), 373.

within the parenchymatous cells, it seems that the fats within the tubercles are not derived from food fats but from the disintegrating tissue cells. Numerous other dyes, however, have been found by DeWitt and others<sup>8</sup> to penetrate tubercles, and this work will be discussed in detail elsewhere. Corper's extensive experiments with copper, both as soluble salts and in the colloidal metal form, showed that this element does not enter tubercles in experimental animals. Presumably even soluble salts of copper combine with the blood proteins and, being thus rendered colloidal, cannot diffuse into the caseous tubercle. As the simple crystalloid, sodium sulphocyanate, is found in the tuberculous tissues in concentration about equal to that in the blood<sup>9</sup> the hypothesis of Wells and Hedenburg that substances enter necrotic tubercles according to the simple laws of diffusion, receives further support, as it does also from the finding of Corper and Arkin,<sup>10</sup> that crystalline salts of arsenic also diffuse readily into tubercles. On the other hand some colloidal dyes do enter tubercles, presumably because they are of somewhat smaller molecular magnitude than the protein colloids that were tested experimentally, or than the colloidal metals and metal-protein colloids that do not enter the tubercles.

The tendency for calcium to enter tubercles is striking, and is exhibited in early stages of necrosis long before either gross or microscopic evidence of calcification can be seen. Thus, in active fatal tuberculosis of guinea pigs, Maver and Wells<sup>11</sup> found that tuberculous organs regularly show much more calcium than normal lesions. This is not a specific feature of tuberculous tissues, however, but probably is exhibited by any necrotic or even partially devitalized tissues, for in degenerating cancers a similar tendency for calcium deposition is seen,<sup>12</sup> as also in other tissues killed or injured by serious interference with circulation; e.g., kidneys after temporary ligation of their blood vessels.<sup>13</sup> This lack of specificity of calcium deposition in tuberculosis, however,

<sup>8</sup> Jour. Infect. Dis., 1913 (12), 68; 1913 (13), 378; 1914 (14), 498.

<sup>9</sup> Corper, Jour. Infect. Dis., 1915 (16), 38.

<sup>10</sup> Jour. Infect. Dis., 1916 (18), 335.

<sup>11</sup> Amer. Rev. Tuberc., 1922 (6), 649.

<sup>12</sup> Clowes and Frisbie, Amer. Jour. Physiol., 1905 (14), 173.

<sup>13</sup> Wells, Holmes and Henry, Jour. Med. Res., 1911 (25), 373.

is not of importance. What is important is the fact that it constitutes a natural demonstration of the fact that certain chemical substances may accumulate in tubercles, thus giving a basis for the hope that some other substance with a more deleterious effect on the tubercle bacilli may be found that will share with calcium in this tendency to accumulate in caseous tubercles.

#### SPECIFIC CHEMOTHERAPY

After this general discussion of the chemical and physical bases of chemotherapeutic action, we may attempt a more detailed account of the experiments which have so far been carried on in the effort to develop a specific chemotherapy of tuberculosis.

In a general sense, chemotherapy is the treatment of a disease by the use of any drug or chemical—the *materia medica* of tuberculosis. Since the time of Ehrlich, however, the term chemotherapy has usually been limited to specific chemotherapy—to treatment by drugs which act on the infectious agent itself, without too seriously affecting the tissues or organs of the host. In the strictest sense, the sense of Ehrlich, chemotherapy has to do with drugs, which, in non-lethal doses, are able to kill all the specific disease organisms within the animal body at a single dose—the “*magna sterilans*” action of Ehrlich, a chemical disinfection of the living body. The animals, however, which he was able to sterilize in this way were infected with certain parasites, trypanosomes and spirochaetes, which have no capsule and which circulate in the blood stream unprotected. This is far from true as regards tubercle bacilli, which are rarely found in the blood stream and which seem to have a waxy envelope; they are also well protected by the mass of tissue called tubercle, and often by phagocytic cells which sometimes seem to serve to protect rather than to destroy these bacteria. Hence the question of direct and immediate bactericidal action in this disease is much more complicated than in the protozoan diseases treated by Ehrlich. A drug, in order to kill the tubercle bacillus, must penetrate the avascular tubercle, often the phagocytic cell, and always the waxy sheath of the bacillus. For these reasons the various indirect methods of attack seem more feasible. However, most of the relatively few experiments which have been carried on with the view of develop-

ing a specific chemotherapy of tuberculosis, have endeavored to attack the problem from the direct or bactericidal or bacteriostatic point of view. Paul Lewis<sup>14</sup> states that the term chemotherapy early came to mean a chemical disinfection of the body or a chemical inhibition of growth in the body, thus aiding the natural defenses of the body. The term is not applied properly to empirical use of drugs, despite the fact that most of our best examples of specific chemotherapy have been provided by the empiricism of primitive peoples, e.g., quinine in malaria, chaulmoogra oil in leprosy, mercury in syphilis.

<sup>14</sup> Harvey Lecture, 1916-1917, p. 112.

## CHAPTER XIV

### SPECIFIC CHEMOTHERAPY WITH ORGANIC COMPOUNDS

#### DYE THERAPY OF TUBERCULOSIS

If we understand chemotherapy to mean chemical disinfection of the body, it is very natural to study first chemical disinfection in the test tube and to determine whether all disinfectants act with equal power on all bacteria. Most disinfectants have been found non-specific in action, but Boer<sup>1</sup> found methyl violet more active against *B. anthracis* than against *B. diphtheriae*, hence partially specific for *B. anthracis*. Ehrlich and Bechhold<sup>2</sup> studied many chemical combinations and found a partial specificity of disinfectant action in certain halogen derivatives of phenol and naphthol. They also found that many apparently trivial chemical modifications could profoundly alter the specific features of disinfection. Most disinfectants are quite toxic in the animal body, but it was soon found that many dyes were quite non-toxic. Many disinfectants are very active *in vitro* but have no action *in vivo*, probably because they are not readily distributed in the animal body so as to come into contact with the cause of the disease. The distribution of dyes throughout the body can be easily determined by color changes. For these reasons, some of the earliest chemotherapeutic experiments in tuberculosis were made with dyes. Lewis found a certain partial specificity of disinfectant action of dyes studied by him,<sup>3</sup> observing that the tri-phenyl methane series of dyes as a group inhibit the growth of pneumococcus and *Staphylococcus aureus* in dilutions which do not inhibit the growth of the tubercle bacillus, while the azo dyes as a group inhibit the growth of tubercle bacilli more readily than

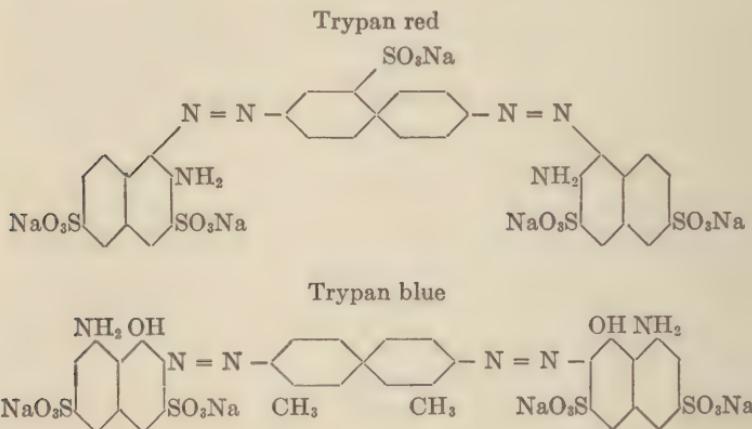
<sup>1</sup> Behring, Gesammelte Abhandlungen, Leipzig, 1893, p. 198.

<sup>2</sup> Bechhold, H., and Ehrlich, P., Zeit. Physiol. Chem., 1906 (47), 173; Bechhold, H., Zeit. f. Hyg., 1909 (64), 113; Bechhold, Desinfection, Paul Ehrlich Festschrift, Jena, 1914, p. 505.

<sup>3</sup> Harvey Lecture, 1916, and Johns Hopkins Hospital Bull., 1917 (28), 120.

that of pneumococcus and *Staphylococcus aureus*. The oxazines and thiazines, to which methylene blue belongs, are more active against the cocci, while the eurhodines, with neutral red as an example, are more active against the tubercle bacilli.

Trypan red, as is well known, is the dye first reported by Ehrlich and Shiga<sup>4</sup> in 1904 as having a magna sterilans action in certain trypanosome infections. Both trypan red and trypan blue are azo dyes having the formulae:



These compounds are readily soluble in water, and when injected into the animal body are taken up by special connective tissue cells called by Goldmann pyrrhol cells, which are so numerous and so filled with deeply colored granules that the connective tissue of the body appears deeply and rather permanently stained. This behavior made it possible for Bowman, Winternitz and Evans<sup>5</sup> to make important observations with these dyes on the histogenesis of tubercles and tuberculous giant cells. Lewis<sup>6</sup> showed that trypan red penetrates to the center of fully formed tubercles in rabbits. DeWitt<sup>7</sup> demonstrated that both trypan blue and trypan red penetrate tubercles in guinea pigs, even the soft caseous substance in the center of tuberculous glands and the large necrotic

<sup>4</sup> Berl. klin. Woch., 1904 (41), 329 and 362.

<sup>5</sup> Cent. f. Bakt. I, Orig., 1912 (65), 403; Jour. Exp. Med., 1914 (19), 283.

<sup>6</sup> Arch. Int. Med., 1912 (10), 68.

<sup>7</sup> Jour. Infect. Dis., 1913 (13), 378.

areas in the spleen and liver being stained deeply blue or red, according to which dye was used, within a few hours after the injection. The tubercle bacilli, however, are poorly or not at all stained by these dyes in the animal body, in the test tube, or when fixed to the slide. The dyes have practically no bactericidal power, since guinea pigs inoculated with tubercle bacilli which had been exposed for twenty-four hours to 1 per cent solutions, quickly developed the disease and died with a local and generalized tuberculosis. They also showed no considerable inhibitory power in the test tube. As Ehrlich and Shiga had found that trypan red failed to kill trypanosomes *in vitro* and yet was very efficient *in vivo*, experiments were carried on to determine if the same might be true in tuberculosis. Guinea pigs were infected with human tuberculosis, treatment was begun at once and the animals kept saturated with the dyes for about two months; they lived somewhat longer than the controls, but died of generalized tuberculosis. Since these dyes penetrate the tubercle so well, it was thought they might be used to carry in some more effective bactericidal agents and trypan blue was therefore combined chemically with silver, with copper, with iron and with mercury. Only the two mercury salts showed increased bactericidal action, the guinea pigs inoculated with tubercle bacilli which had been exposed to these developed neither local nor general tuberculosis. Treatment of tuberculous guinea pigs with these metal compounds of trypan blue, copper, iron and silver salts, failed to affect the progress of the disease, while the animals treated with the mercury salt lived somewhat longer and had much less tuberculous involvement. Hence trypan blue and trypan red, either as the sodium salts or as any one of the metal salts used in these experiments have failed to kill the tubercle bacilli either in the test tube or in the animal body, or to cure experimental tuberculosis in guinea pigs.

Lewis tested many chemical combinations of trypan blue and trypan red, with iodine, phenol and certain other substances. As DeWitt<sup>8</sup> had found with her compounds of trypan blue and trypan red, none of these compounds had any therapeutic effect, though some seemed to influence the vigor and rate of formation of

<sup>8</sup> Jour. Infect. Dis., 1914 (14), 498.

blood vessels and connective tissue around the tubercle in the rabbit's eye. He then combined the dye, Niagara blue—2 B, with iodin, phenol and certain fatty acids. The resulting compounds were definite chemical compounds, usually dyes, and different from the original substance. The inhibitory power was usually increased by the chemical combinations, but the power to enter the tubercle was diminished or completely lost. One compound of the dye with formic acid showed great increase of inhibitory power without loss of power to stain the tuberculous tissues. Several diazo creosote dyes were made, violet blue in color, which inhibit the growth of the tubercle bacillus at  $\frac{1}{100,000}$  and penetrate the tubercles in the living animal with moderate intensity, and which extended the life of the tuberculous guinea pig in some cases. A number of dyes or dye compounds have thus been found or developed which penetrate and stain the tubercle bacillus and which penetrate the tubercle, sometimes more or less specifically. None, however, has cured the disease in guinea pigs.

Because of the unusual fat content of tubercle bacilli, it was early thought that fat soluble and fat staining dyes might have a specific action on the tubercle bacillus and on tuberculosis. Hope Sherman<sup>9</sup> showed that fat dyes do not readily stain tubercle bacilli, while basic fuchsin, eosin, and methylene blue, which are not classed as fat dyes, stain them deeply in a short time. Corper<sup>10</sup> found no inhibitory action of fat dyes on the growth of tubercle bacilli, no power to penetrate the tubercle in the living animal and no therapeutic effect on experimental tuberculosis in guinea pigs.

The only other dye that has been used to any considerable extent in chemotherapeutic experiments in tuberculosis is methylene blue in its various modifications. DeWitt<sup>11</sup> in 1912 showed that methylene blue easily penetrated the tubercle bacillus *in vitro* and the tubercle in the living body, where the dye was reduced, but could be re-oxidized. Tubercle bacilli were not easily stained in the living animal body by methylene blue, but were completely inhibited in cultures containing about  $\frac{1}{10,000}$  and killed in some cases in 1 per cent solutions though weaker solutions were not

<sup>9</sup> Jour. Infect. Dis., 1912 (12), 249.

<sup>10</sup> Jour. Infect. Dis., 1912 (12), 373.

<sup>11</sup> Jour. Infect. Dis., 1913 (13), 378.

tested. In those animals in which tuberculosis developed after the cultures used for inoculation had been exposed to 1 per cent methylene blue, the development was much delayed and the degree of distribution was less than in the controls. But even though a dye penetrates the tubercle and though it has good bactericidal or bacteriostatic action *in vitro*, we must know what action it will have in the body. We must take into consideration the biologic factor.

The animal generally used for these experiments is the guinea pig, which is so susceptible to tuberculosis that the problem of curing the disease is extremely difficult. Because of this we may feel quite sure that a drug which cures the disease in the guinea pig, or even influences it very favorably, would more certainly be beneficial in the much less susceptible human being. For this reason and also for economic reasons, the guinea pig is chosen. For similar reasons, the subcutaneous method of inoculation is the method generally used, although Lewis in most of his work used rabbits and conjunctival inoculation in order that the penetration of the drug and its action could be constantly watched.

In several communications between 1912 and 1920, Frau Gräfin von Linden described the therapeutic results which she obtained in 6 guinea pigs inoculated with tuberculosis and treated with methylene blue iodide. She used daily subcutaneous injections of 0.2 to 0.4 cc. of a 1-1000 solution of medicinal methylene blue and states that this treatment healed the inoculation abscess in six weeks, delayed the involvement of local inguinal glands from thirteen days in the controls to thirty-seven days in the treated animals, when they enlarged more slowly and to less extent than in the controls and in some animals sclerotized, in others suppurated, evacuated and then healed. The temperature curve was more nearly normal; the weights remained high longer and life was prolonged to nearly double the average life of the controls. All organs were much less involved than in the untreated animals. DeWitt<sup>11</sup> in 1913 published a report of a number of experiments on the treatment of tuberculosis in guinea pigs with methylene blue and derived dyes. Table 1 will give the average results, the degree of tuberculosis in the organs being roughly estimated in percentages, on a basis of 100 per cent for the controls.

TABLE I

The results are not quite so striking as those shown by von Linden, but they do show a much less extensive disease in the treated animals than in the controls. In the main, the duration of life was nearly the same in the treated as in the untreated animals, but sometimes it was less and sometimes more. Average duration of life does not have great value, since there are often very considerable differences between the maximum and the minimum, so that a single long lived animal may very greatly affect the average. The animals treated with methylene blue, iod-methylene blue, and the double salts of mercuric chloride with methylene blue, methylene green and iod-methylene green showed a rather definitely longer duration of life than that of the controls. The extent of the disease was considerably less in the treated animals than in the controls. However, though the methylene blues may have somewhat modified the disease, these reports show that in no single case was the disease cured by any of the methylene blues.

None of the dyes so far tested, then, has shown any definite therapeutic power in experimental tuberculosis in guinea pigs beyond some prolongation of life, and some lessening of the severity of the disease, although the penetrating and bactericidal or bacteriostatic power were good. This failure may have been due to a number of causes. They may have been too toxic to make it feasible to use doses large enough to secure and maintain a bactericidal concentration in the tuberculous tissue. The dye may be too rapidly excreted to maintain the desired concentration. The dyes are mostly rather complex chemical structures which may break down or undergo some chemical change in the body which may alter the disinfectant power. Thus, Pfeiffer<sup>12</sup> found that tuberculous patients possess an exceptional power to destroy methylene blue. Lung tissue possessed the greatest ability to destroy the dye. The blood from cases of tuberculosis changed the dye more rapidly than other bloods, while the serums did not differ. Within six to ten hours, 3 of the 8 leuco-products of methylene blue were no longer excreted.

It is still possible that these easily diffusible dyes may be used as carriers for more efficient therapeutic substances, which may

<sup>12</sup> Beitr. Klin. Tuberk., 1921 (47), 46.

reach in this way the tuberculous tissue which they could not otherwise attain. The subject of these interesting chemotherapeutic agents has by no means been exhausted.

#### CREOSOTE, GUAIACOL AND THEIR DERIVATIVES

According to Nolen<sup>13</sup> creosote was first introduced into the therapeutics of tuberculosis in 1830 on the recommendation of Reichenbach.<sup>14</sup> But the results were unsatisfactory and it was dropped until 1877 when Bouchard, according to Weismayr, after using it in 93 cases with generally favorable results, stated that it lessened expectoration, cough, fever and night sweats, improved the appetite and general condition and the physical signs in the diseased lungs. Since that time, creosote and allied preparations have been used more and more in tuberculosis and probably hold first place among the countless drugs used in that disease, not so much as specific, bactericidal agents as with the idea that they have a favorable influence on the signs and symptoms of the disease. Perhaps as good a summarization of their status as any was given by Lawrason Brown,<sup>15</sup> who says that creosote and its derivatives are

the most used of false specifics. They have never been proved to exert any action on the tuberculous process but in some patients have an almost specific action upon the accompanying secondary infection of the lungs, such as simple bronchitis. They also exert a very stimulating effect upon the bronchial mucous membrane during their excretion through it.

The factories have constantly made new derivatives until Nolen in 1914 named 30 compounds and derivatives of creosote and guaiacol. The question as to the clinical value of these drugs has never been settled. Kober says, "Very little that is conclusive can be said concerning the usefulness of these preparations." Bandelier-Ropke's "Die Klinik der Tuberkulose" concludes that "creosote and guaiacol preparations are not internal disinfectants, but in certain cases they stimulate the appetite and improve digestion. Their routine use is therefore by no means justified."

<sup>13</sup> Brauer, Schröder and Blumenfeld Handbuch der Tuberkulose, 1914 (2), 179.

<sup>14</sup> Schmidt's Jahrbuch, 1830 (2), 129.

<sup>15</sup> Klebs, Tuberculosis. New York, D. Appleton and Co., 1909.

General clinical experience is the only extensive source of information concerning the value of these drugs, and this is of course uncontrolled, hence conflicting and of little value. The most that can be said is that the use of creosote therapy in tuberculosis has persisted so long and so widely that it seems probable that some beneficial results have been observed and we may suspect that thorough experimentation may bring to light the limitations and principles on which it depends.

Since bactericidal action, according to our generally accepted definition, lies at the basis of specific chemotherapy, it seems worth while to quote in full the compilation of the literature concerning the bactericidal action of these compounds both *in vitro* and *in vivo* given by DeWitt, Suyenaga and Wells.<sup>16</sup>

Bouchard is quoted by Weismayr as having found that 0.8 per 1,000 strength of creosote in glycerol bouillon retards the growth of tubercle bacilli, and 0.5 per 1000 is effective in blood serum. In daily doses of 0.25 grams per kilo it caused immunity to tuberculosis in rabbits, so that animals killed after three months showed no lesions. We regret that we have not been able to find the origin of this statement, for it is perhaps the most definite one in the literature.

On the other hand, Cornet<sup>17</sup> reported that he inoculated 7 guinea-pigs with tuberculosis after they had been given 0.02 gram creosote daily per catheter into the stomach for about a month, and continued after infection, but they all died of tuberculosis as did the control animals. This dose he estimated as corresponding to 2.0 grams creosote per day in a man. He also quotes Schüller as obtaining positive results and Sormani and Pallacani as obtaining negative results by causing infected guinea-pigs to inhale creosote.

Guttmann<sup>18</sup> studied the inhibition of growth of different species of bacteria on gelatin containing creosote, which he found to be more effective than phenol. Of 18 species of bacteria, 12 failed to grow when the gelatin contained one part in 2000 (including 4 not growing at 1:4000), and 5 of the remaining 6 failed to grow when the concentration was 1:1000. With 17 species on gelatin containing phenol, 12 grew when the concentration was 1:2000. Tubercle bacilli were partly inhibited by creosote 1:4000 and even 1:16000, but it required 1:2000 to prevent growth entirely. He then speculated as to the possibility of obtaining a concentration of 1:2000 of creosote in the human body, and found that this could not be done, wherefore he was doubtful as to the efficiency of creosote in tuberculosis—a speculation that has been quoted extensively in literature on tuberculosis.

<sup>16</sup> Jour. Infect. Dis., 1920 (27), 115.

<sup>17</sup> Ztschr. f. Hyg. u. Infektionskr., 1888, 5, p. 124.

<sup>18</sup> Ztschr. klin. Med., 1888, 13, p. 488.

Fraenkel<sup>19</sup> corroborated in 1889 the statement that cresols are stronger antiseptics than phenol. He quotes von Behring's estimate that one-sixth the amount of an antiseptic found to be inhibiting for bacteria represents approximately the lethal dose for an animal. In accordance therewith, Fraenkel found that 1:3000 cresol sulfate is inhibiting to bacteria (species not stated) which would correspond on this basis to  $\frac{2}{6}$  gram in a 600 grams pig, an amount found fatal although a slightly smaller dose was not fatal.

Winkler<sup>20</sup> exposed agar plate cultures of tubercle bacilli to vapor from a mixture of guaiacol and iodoform for eight days and found that the material became noninfectious. Injection of this mixture into animals did not save them from tuberculosis, and serum of rabbits injected with the antiseptic had no effect on infection with tubercle bacilli.

Villa is quoted by von Weismayr as having found that guaiacol prevents growth of streptococci in a dilution of 1:1000, and kills in this dilution in 16 minutes, and in dilution of 1:100 in two minutes.

Hammer<sup>21</sup> found that paracresol is equal to orthocresol in bactericidal power against staphylococci and typhoid bacilli, but more toxic. Phenol was less strongly bactericidal and more toxic than either.

Several authors quote Shaw<sup>22</sup> as having demonstrated that guaiacol is ineffective in infections of animals, but the original article shows that he merely inoculated two rabbits with *B. pyocyaneus*, and injected one with 20 cc. of a 1:200 guaiacol solution (the lethal dose of which is 25 cc.). This animal died in eighteen hours and the control in twenty-six hours. There is no other experimental evidence in this much quoted article.

One of the most important contributions to the subject of creosote therapy is that of Bechhold and Ehrlich,<sup>2</sup> who (using especially diphtheria bacilli for their tests) developed many new and fundamental facts in relation to the influence of various modifications of the phenol derivatives on their bactericidal and physiologic action. The chief conclusions were:

1. Introduction of halogens into phenol increases the disinfectant action in proportion to the number of halogen atoms introduced<sup>23</sup> (e.g., 1 mol. pentabrom phenol has the same action on diphtheria bacilli as 500 mol. phenol).

2. Alkyl groups introduced into phenols or halogen phenols increase their disinfectant action. (Tribrom-m-xylenol is 20 times as active as tribrom phenol; tetrabrom-o-cresol is 16 times as active as tetrachlor phenol.)

3. Union of 2 phenols or halogen phenols, either directly or through  $\text{CH}_2$ ,  $\text{CHOH}$ ,  $\text{CHOCH}_3$  or  $\text{CHOC}_2\text{H}_5$  groups, increases activity. Thus, tetra-

<sup>19</sup> Ztschr. f. Hyg. u. Infektionskr., 1889, 6, p. 521.

<sup>20</sup> Deut. med. Wchnschr., 1893, 19, p. 781.

<sup>21</sup> Hyg. Rundschau, 1899, 9, p. 1017.

<sup>22</sup> Jour. of Hygiene, 1903, 3, p. 159.

<sup>23</sup> In a Patentschrift, Dammann, in 1889, also mentions this effect of halogens; quoted by Schottelius, Arch. f. Hyg. 1913, 82, p. 76.

brom-o-cresol inhibits the growth of diphtheria bacilli in a dilution of 1:200,000, while tetrabrom-o-biphenol inhibits when diluted to 1:640,000.

4. Union of 2 phenols through CO or SO<sub>2</sub> decreases activity.
5. Introduction of COOH into the nucleus decreases activity.
6. Halogens introduced into phenols at first reduce toxicity, but the trihalogens have about the same toxicity as the unhalogenized substance, and tetra- and penta-halogen compounds are more toxic. However, the spasmodic action of the phenols is reduced in proportion to the number of halogen atoms. CH<sub>3</sub> groups compensate or neutralize the toxicity introduced by the halogens.

Of the compounds developed in this study the most effective were:

Tetrabrom-o-cresol, which has but little toxicity yet inhibits growth of diphtheria bacilli diluted to 1:200,000, and in 1 per cent solution kills them in less than two minutes. It compares in activity with phenol in the ratio of 1,000:0.9.

Tetrabrom-o-biphenol (and the corresponding Cl compounds) which is more toxic but inhibits growth at a dilution of 1:640,000.

Hexabrom-diphenyl carbinol, practically nontoxic, inhibits growth at 1:200,000; kills in twenty-four hours in dilution of 1:320,000 and kills in ten to fifteen minutes at 1:1000. Compares with phenol as 1000 to 0.6 in respect to action on diphtheria bacilli, although less effective against "water bacteria" than phenol.

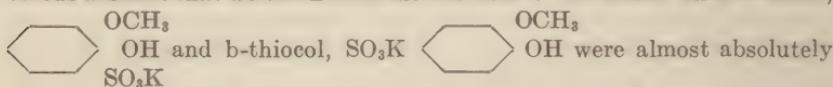
Although these substances did not precipitate proteins they were ineffective against diphtheria bacilli in serum, and on this basis the authors explain their failure to influence favorably diphtheria infection in animals. Unfortunately, they give no details as to the methods used in conducting these experiments.

This article was followed by a report by Bechhold<sup>2</sup> under the title of "Halbspezifische chemische Desinfektionsmittel" in which is emphasized the fact that the effect of a given chemical on one species of bacteria may not be duplicated with another species, and hence general laws covering the influence of various modifications of a substance on its bactericidal action cannot be deduced from limited observations. Thus, in the previous article it was stated that the introduction of halogen atoms into phenols increases the disinfectant action somewhat in proportion to the number of added halogens. But Bechhold finds that against staphylococci, streptococci and diphtheria bacilli the maximum disinfectant power is shown by tribrom- $\beta$ -naphthol, as compared with either mono- and di- or tetra- and penta-brom- $\beta$  naphthol. On the other hand, against paratyphoid bacilli the activity is constant as halogens are added to dibrom or dichlor, and decreases with three or more halogen atoms. The "semi-specificity" of this class of disinfectants is shown by several examples. Thus, tetrabrom-p-biphenol and tribrombikresol are very active disinfectants for staphylococci, but against colon bacilli they are less effective than lysol. While tri- or tetrabrom- $\beta$ -naphthol, tetrabrom-o-cresol and tetrachlor-1-biphenol have a considerable disinfectant action even on anthrax spores, they as

well as some others of the higher halogen phenols, are practically inactive against tubercle bacilli. Tetrabrom-o-cresol, hexabromdioxydiphenyl-carbinol, tetrachlor-o-biphenol, tetrabrom-biphenol and tribromcresol, in 1 per cent solution for 2 hours with an emulsion of human tubercle bacilli did not impair their infectivity for animals. Tri-tetrabrom- $\beta$ -naphthol acted in 2.5 per cent solution on tubercle bacilli for 25 hours without effect, while a 5 per cent lysol solution (containing 2.5 per cent of cresol) kills tubercle bacilli in four and one-half to eight hours. Hence all these disinfectants which are much more actively destructive of staphylococci than lysol, are much less effective than lysol against tubercle bacilli.

As far as we can learn, the leads given in these articles have not been followed much farther, either in Ehrlich's laboratory or elsewhere. Leubenheimer<sup>24</sup> has established anew the general applicability of the principle of the effect of halogenized phenols to bacteria, and also demonstrated for different xylenes a high bactericidal action (Schottelius).<sup>25</sup>

Heukeshoven<sup>26</sup> has made the most extensive study of the action of thiocol, and one of the few studies of the effect of cresote derivatives on tuberculous animals that we can find in the literature. He found that a-thiocol,



without inhibiting effect on staphylococci, anthrax and *B. pyocyaneus*, for all these grew in asparagin glucose agar containing from 1 to 5 per cent of these compounds; while guaiacol carbonate, being used merely as a suspension, had little more effect, but K-guaiacolate prevented growth of staphylococci in 0.5 per cent (the lowest concentration tried), and inhibited anthrax and pyocyaneus at 2 per cent but not at 1 per cent.

The animal experiments were performed with four series of rabbits inoculated with tubercle bacilli (origin not stated) in the eye. In each series were nine rabbits, one serving as a control. They received daily doses of 0.5 gram of each of the 4 guaiacol derivatives mentioned above, by means of a catheter. In one series the drug was given for fourteen days before infection and not afterward; in the second the treatment was continued; in the third there was no preliminary treatment, but treatment continued after the infection; in the fourth treatment was not begun until four weeks after the inoculation. The result was, in brief, that in the rabbits receiving thiocol-a, 2 recovered completely and in none was the disease disseminated through the body; with thiocol-b, none recovered, in 6 there was no dissemination, and in 2 there was dissemination; with potassium guaiacolate, in 5 of 8 the tuberculosis was disseminated; with guaiacol

<sup>24</sup> Phenol und seine Derivate, Berlin, 1909; quoted by Schottelius.

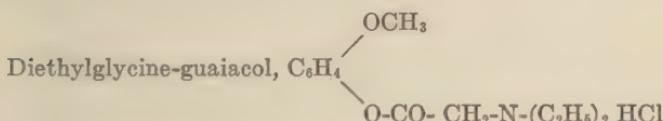
<sup>25</sup> München. med. Wchnschr., 1912, 59, p. 2674.

<sup>26</sup> Experimentelles über die Wirkung des "Thiocols" bei der Tuberkulose. R. Heukeshoven, Inaug. Dissert., Bern, 1899.

carbonate it was disseminated in 6 of 8, and in the 4 controls. He also found increase of weight in animals given thiocol-a or guaiacol carbonate; potassium guaiacolate had a deleterious effect on the animals and thiocol-b had no effect. As it has been found that thiocol is excreted unchanged, and as it seems to be devoid of bactericidal properties (although not tested on tubercle bacilli) these favorable effects are difficult to explain.

Lynosol, which differs from thiocol in having Ca in place of K, is said by Takenaka<sup>27</sup> to be as strongly bactericidal for tubercle bacilli as creosote and guaiacol, which all, he says, inhibit growth at concentrations over 1:10,000.

Lubowski<sup>28</sup> has reviewed the abundant literature on thiocol to the middle of 1904, but this literature contains no work that seems to be accurate except that of Heukeshoven, nor have we found any contributions of importance since that date.



known commercially under the name Guiasanol, has been produced and described by Einhorn<sup>29</sup> as having many therapeutic advantages. Gastric irritation is avoided because the free base is not formed until the HCl is split off in the intestinal alkalies, and free guaiacol is eventually liberated since it is found in the urine.<sup>30</sup> The toxicity is low, rabbits being uninjured by 2 grams subcutaneously, or 4 grams by stomach, and 2 per cent solutions are not irritating. Buchner tested the bactericidal action of guiasanol, finding it low *in vitro*, for it inhibited the growth of *B. coli*, *B. pyocyanus*, *B. proteus* and *S. pyogenes aureus* only in concentrations of 1:50 or 1:100. If it is correct that guiasanol readily liberates guaiacol in the body, the low bactericidal power *in vitro* is of little significance, and Einhorn claims that it is efficient as a local antiseptic in ulcerating cancer and tuberculous enteritis.

A few other incidental references complete the list.

Burow<sup>31</sup> reported that a 3 per cent aqueous solution of sodium or potassium guaiacolate containing 0.01 per cent potassium arsenite prevented growth of tubercle bacilli, although without the arsenic the guaiacol was ineffective. He also stated that the blood of a rabbit treated with this mixture would not support growth of tubercle bacilli, and that guinea-pigs

<sup>27</sup> Japanese Jour. Tuberc. Kekkakau Zassi, 1919, 2, No. 1.

<sup>28</sup> Allg. Med. Centr. Ztg., 1905, 74, pp. 337, 356, 396.

<sup>29</sup> München. med. Wchnschr., 1900, 47, p. 10.

<sup>30</sup> Concerning absorption and elimination of guaiacol see Eschele, Ztschr. klin. Med., 1896, 29, p. 197.

<sup>31</sup> München. med. Wchnschr., 1910, 57, p. 1792.

and rabbits thus treated resisted tuberculous infection. These results he attributed chiefly to the arsenic. This work, the report of which does not give a convincing impression, was repeated by Nürnberg,<sup>32</sup> who found no effects produced in either cultures or animals by the guaiacol-arsenic mixture of Burow, or by 0.01 gram Na guaiacolate in glycerin-agar, the concentration not being stated.

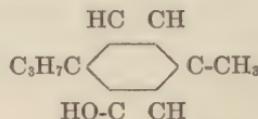
J. Naberly<sup>33</sup> reported favorable clinical effects with a "new guaiacol chloriodid compound," the exact nature of which is not given. As there is no experimental evidence in regard to this compound, it is mentioned only because it has some possible relation to Ehrlich's observations. The Lancet laboratory examined this compound and found the iodin and chlorin nearly all in chemical union.

Cooper<sup>34</sup> reports an extensive study of creosote and allied substances, particularly with reference to the disinfectant action of various soap solutions in surgery and disinfection work. The chief facts developed of interest in this connection are:

Using the Rideal-Walker method as modified by Chick and Martin, the phenol coefficient of the cresols in pure aqueous solution was found to be:

|                      | B. TYPHOSUS | S. PYOGENES<br>AUREUS |
|----------------------|-------------|-----------------------|
| Ortho cresol.....    | 2.6         | 2.1                   |
| Meta cresol.....     | 2.6         | 2.0                   |
| Para cresol.....     | 2.6         | 2.4                   |
| Thymol.....          | 25.0        |                       |
| "Cresylic acid"..... |             | 2.2                   |

Therefore, as shown by thymol,



alkyl groups in the benzene ring may increase greatly the germicidal action, which accords with Bechhold's and Ehrlich's statements, and with the observation made by Koch in 1881.

On the other hand, the introduction of a second OH group into phenol decreases bactericidal action. Thus, for typhoid bacilli in water the phenol coefficient of various OH compounds was: resorcin, 0.3; pyrocatechin, 0.5; hydroquinone, 1.0; pyrogallol, 0.77; phloroglucin, less than 0.35.

Quinone, O  O, however, had a phenol coefficient for staphylococci of 10, whereas acetone was less than 0.075.

<sup>32</sup> Münch. med. Woch., 1911 (58), 2669.

<sup>33</sup> Lancet, 1913, 2, p. 285.

<sup>34</sup> Brit. Med. Jour., 1912, 1, p. 1234.

Krauss,<sup>35</sup> in describing several new compounds of trypan red, gives the method of making guaiacol trypan red and iodo-guaiacol trypan red. He says nothing concerning their activity, but Paul Lewis in a brief note published elsewhere<sup>36</sup> states that no effects had been obtained in experimental tuberculosis with any of their trypan red compounds.

DeWitt, Suyenaga and Wells,<sup>16</sup> in order to determine whether there is any reason for believing that the various members of the guaiacol series should be expected to have any direct action on tuberculosis, tested the inhibitory or bacteriostatic action on human tubercle bacilli *in vitro* of creosote, guaiacol, o-cresol, m-cresol, p-cresol, thymol, creosol, resorcin, thiocol, styracol, sodium guaiacolate, guaiacol cacodylate, hydroquinone and pyrocatechin. Complete inhibition was effected by 0.1 per cent creosote or guaiacol; by 0.05 per cent of creosol; by 0.01 per cent of the three cresols, thymol and resorcin; by 0.1 per cent of guaiacol cacodylate, and hydroquinone; by 0.05 per cent of pyrocatechin; by 0.1 per cent of sodium guaiacolate, while not even 1 per cent of thiocol or styracol showed any inhibitory effect on the growth of the human tubercle bacillus on glycerol agar.

Bactericidal tests, in which the capacity to grow on agar after exposure of clumps of tubercle bacilli to the antiseptic was the measure of action, showed that the bactericidal power of these substances is low. Exposure to even 1 per cent solutions of pyrocatechin, hydroquinone, resorcin, and 0.5 per cent solution of creosol, for periods from ten minutes to forty-eight hours, entirely fails to kill human tubercle bacilli. Metacresol and paracresol kill in 1 per cent concentration after exposure for one hour, but not after ten minutes, while orthocresol reduces growth after one hour, and kills in six hours. Thymol kills even in ten minutes at 1 per cent concentration, but 0.1 per cent concentration does not kill even in forty-eight hours. Weaker concentrations of all these antiseptics were, of course, without bactericidal effect.

A bactericidal test was made with the tubercle bacilli exposed to antiseptics when in a thin layer on the surface of garnets, and viability determined by inoculating guinea-pigs with the treated bacilli washed from the garnets. Resorcin in 1 per cent solution

<sup>35</sup> Jour. Amer. Chem. Soc., 1914, 36, p. 960.

<sup>36</sup> Jour. Pharm. and Exper. Therap., 1914, 4, p. 353.

killed the tubercle bacilli only after twenty-four hours' exposure. Orthocresol killed in 1 per cent concentration even in twenty minutes, but 0.5 per cent did not kill even in twenty-four hours. Creosote and guaiacol both killed most of the bacilli in 0.5 per cent concentration, even in twenty minute exposure, but 0.1 per cent concentration was not bactericidal in twenty-four hours. Thymol was bactericidal in 0.1 per cent concentration, even in an exposure of twenty minutes, but 0.01 per cent was not bactericidal in an exposure of twenty-four hours.

Therapeutic tests were made on guinea-pigs injected subcutaneously with two strains of human tubercle bacilli, one highly virulent and the other much less so. The animals were then given several doses of the drug by the intracardiac, intramuscular and subcutaneous routes, and daily feedings of pills containing the drugs, the following being tested: Creosote, guaiacol, creosol, thiocol, styracol, orthocresol, metacresol, paracresol and thymol. In all, 106 guinea pigs were thus treated for long enough periods to observe the results (besides the control experiments). Apparently the treated animals that had been infected with the less virulent tubercle bacilli showed more active tuberculosis than the untreated controls, as if the treatment had lowered their resistance or stimulated the bacilli. With the more virulent bacilli, the extent of the disease was perhaps slightly less in the treated animals, probably because they commonly died a little sooner than the controls.

Our experiments show that substances of the creosote series do not possess a high bactericidal power for the tubercle bacillus *in vitro*, and apparently not *in vivo*. This result is not surprising in view of the observations of DeWitt and Sherman<sup>37</sup> that tubercle bacilli are rather less susceptible to fat-soluble, and more susceptible to water-soluble antiseptics, than bacteria less rich in fat than the tubercle bacillus. Also from their observation that fat-soluble dyes do not readily penetrate tubercle bacilli, while certain fat-insoluble dyes (e.g., methylene blue) stain them well. Apparently the lipin-rich character of the tubercle bacilli does not make them vulnerable to fat-soluble antiseptics, but rather the reverse.

<sup>37</sup> Jour. Infec. Dis., 1914, 15, p. 245.

The figures given above for the bactericidal power of the guaiacol group may be compared with those obtained by DeWitt and Sherman, using similar methods. Phenol kills in 1 per cent concentration, and shows some effect in 0.1 per cent concentration. Formaldehyde kills in 1 per cent in one hour, in 0.1 per cent in twenty-four hours. Ethyl alcohol, 25 per cent kills in one hour or less. Acetone, chloroform and ether have little or no tuberculocidal action; toluene and iodin show slight influence. Mercuric chlorid kills in 0.001 per cent in twenty-four hours, 0.1 per cent in one hour; gold chlorid in 0.005 per cent kills in twenty-four hours, as do 0.25 per cent silver nitrate, 0.1 per cent gold tricyanid and 5 per cent copper chlorid. Evidently creosote, guaiacol and the cresols, have about the same tuberculocidal power as phenol, which is distinctly not high. The dihydroxy phenols, resorcin, hydroquinone and pyrocatechin, seem to be less active than the monhydroxy phenol. Thymol was, in all experiments, distinctly, although only slightly, more bactericidal than the other substances tested. This agrees with the statement of Bechhold and Ehrlich<sup>2</sup> that addition of alkyl groups to phenols increases their disinfectant action.

The failure to observe any beneficial therapeutic effect on tuberculous guinea-pigs is, in view of the low bactericidal power of the substances tested, to be expected. It does not mean, however, that these substances may not have value in open tuberculous infections in man in which other bacteria than *B. tuberculosis* are involved. But it does substantiate the opinion that seems to have been generally reached by careful clinical observers, that creosote and guaiacol do not have a specific action on tuberculous infection.

#### FATS AND FATTY ACIDS

Among the favorite *old* remedies for pulmonary tuberculosis used by physicians was cod liver oil with no very definite idea of why it had any value. Saugman<sup>38</sup> quotes Schepelern in 1900 and Schtangeieff in 1896 as ascribing to it a marked, direct healing influence on fever. Saugman is inclined to agree with this, but

<sup>38</sup> Fieber und Nachtschweisse, Brauer, Schröder, Blumenthal's Handbuch der Tuberkulose, 1914, (2), 315.

states that in any case, we have in cod liver oil an easily assimilable fat. Regarding fats as food in tuberculosis, Schröder<sup>39</sup> quotes Weigert as having proved that overfeeding swine with fat increased their resistance to tuberculosis; and Vogt as having seen greater immunity to tuberculosis in fat-nourished children. Since fatty foods are often not well taken persistently, the "most important substitute is cod liver oil to which has been ascribed an almost specific influence in scrofulous and tuberculous diseases." Schröder states that the salts of cod liver oil are less effective and advises the pure oil. "Lipanin," containing 6 per cent olive oil or pure oil of sesame, is given in the same way and in the same dosage. It may be noted that in this earlier work, the cod liver oil was given not only on account of its food value as a fat, but also with the idea that it increased the resistance to the disease.

It was stated, however, by Miller<sup>40</sup> in 1916 that the addition of sperm oil to media on which tubercle bacilli were being grown caused rapid degenerative changes in the bacilli; they became granular and lost their staining peculiarities. Some experiments in this laboratory have confirmed Miller's results. Fontes<sup>41</sup> states that addition of 1 per cent cod liver oil or chaulmoogra oil or beef gall to ordinary broth culture medium prevents the development of cultures of tubercle bacilli. This impeding action was prevented by adding 2 per cent gelose to the culture medium, solidifying it. The oil in the fluid medium did not lose this inhibiting power when filtered through porcelain but did lose it when shaken with kaolin. Fontes thinks the bacilli absorb the fat particles which form an isolating envelope, insulating the bacilli from the nutritive medium and from the oxygen in the broth. If this theory be true, it will refute the idea of any specificity of the effects produced *in vitro* by any one of these oils for the tubercle or other acid fast bacilli.

Beneficial effects have been cited by so many clinicians from the use in tuberculosis of cod liver oil, which are apparently wanting after the use of the salts of the fatty acids, that it may be believed

<sup>39</sup> Brauer, Schröder, Blumenthal's Handbuch der Tuberkulose, 1914 (2), 105.

<sup>40</sup> Jour. Path. and Bact., 1916 (20), 395.

<sup>41</sup> Brazil medico, Rio di Janeiro, 1921 (35), 95.

that the difference lies in the absence in the latter of the vitamines to which probably much of the value of the oil is due. Recent studies on the specific therapeutic effect of cod liver oil in rickets have brought out the remarkable fact that cod liver oil and heliotherapy have identical effects both on the progress of the disease and on the metabolism of the subject, there being an accelerated rate of recalcification and an increase in the inorganic phosphorus of the blood.<sup>42</sup> Since heliotherapy and cod liver oil both benefit tuberculosis, especially the surgical forms, it seems probable that their influence here may be similar to their influence in rickets. We do not as yet know whether the active agent in this respect is the same as the "vitamine A" which favors growth and absence of which leads to keratomalacia.

### *Chaulmoogra oil*

Walker and Sweeney<sup>43</sup> have found that chaulmoogra oil contains substances about 100 times more bactericidal than phenol and specific for the acid fast group of bacteria only. These substances are the fatty acids of the chaulmoogric series, chaulmoogric and hydnocarpic acids and possibly lower isomers of this series. They found that the fatty acids of cod liver oil do not possess the specific activity of the chaulmoogric oil series. Lindenberg and Pestana,<sup>44</sup> however, reported that the active principle of chaulmoogra oil was in its acids and that the non-saturated fatty acids of chaulmoogra and other oils possess the property of inhibiting the growth of acid-resisting bacteria and check the proliferation of the leprosy bacillus and of the tubercle bacillus. To this they ascribe the benefit from chaulmoogra oil in leprosy and from cod liver oil in tuberculosis. They claim that these oils act as direct chemotherapeutic agents and not as stimulants for phagocytosis nor as tonics. They tested various oils with cultures of various acid-resistant bacteria and found chaulmoogra oil from *Taraktogenos Kurzii* most potent and the only one which constantly prevented development of the various microorganisms.

<sup>42</sup> See A. F. Hess, Jour. Amer. Med. Assoc., 1922 (78), 1177; Janet Clark, Physiol. Reviews, 1922 (2), 277.

<sup>43</sup> Jour. Infect. Dis., 1920 (26), 238.

<sup>44</sup> Brazil Medico Rio de Janeiro, 1920 (34), 603.

Hollman,<sup>45</sup> after using chaulmoogra oil alone for twelve years in the treatment of leprosy with great success, tried the fatty acid fractions and found them superior to the oil. Rogers,<sup>46</sup> after finding sodium hydnocarpate most active in leprosy, used the same method for extracting fatty acids of cod liver oil with ether and then making a sodium salt, which he called sodium morrhuate. He had tried the sodium hydnocarpate on tuberculous animals but the experiment failed "for want of virulent bovine tuberculosis culture." The sodium morrhuate acts in leprosy much as does the sodium hydnocarpate, showing no specificity of either salt for leprosy. Rogers thinks that the sodium salts of the unsaturated fatty acids of both the oils act in the same way on the coating of the acid fast bacilli, that of the tubercle bacillus having been shown to contain palmitic and other unsaturated fatty acids. During a year's testing of sodium morrhuate in clinical cases of pulmonary tuberculosis, morphologic changes were noted in the tubercle bacilli in the sputum. As success has attended the use of sodium morrhuate in lupus, Rogers<sup>47</sup> thinks there is ground for hope that we may in the future be successful in treating in this way the more chronic tuberculous cases, such as surgical tuberculosis. He is, however, doubtful about the advisability of using it in pulmonary tuberculosis, until further evidence is available regarding its value in more localized and superficial processes where any reactions may be seen and easily watched.

Walker and Sweeney<sup>48</sup> state that acid fast bacilli which will grow submerged in broth are completely inhibited by dilutions of sodium chaulmoograte of from 1:80,000 to 1:140,000 and complete bactericidal action was obtained at 1:20,000 after twenty-four hours' exposure in the incubator. They think that this inhibitory and bactericidal activity is confined to the carbon ring structure found in chaulmoogric acid and not in the fatty acids from cod liver oil. Walker's preliminary experiments with these compounds as chemotherapeutic agents in experimental tuberculosis of rabbits and guinea pigs seem to show that they have some value, since the average duration of life of the treated animals was

<sup>45</sup> Jour. Cutaneous Dis., 1919 (37), 367.

<sup>46</sup> Indian Med. Gazette, 1919 (54), 165; Brit. Med. Jour., 1919 (2), 147.

<sup>47</sup> The Practitioner, 1921 (107), 77.

greater than of the untreated. The degree of progress of the disease was also often less in the treated animals than in the untreated. Walker announces that other experiments with a larger number of animals are under way.

Culpepper and Ableson<sup>48</sup> have tested the ethyl esters of the four acid fractions, finding them irritating after intraperitoneal injection and toxic in 1 cc. doses of 1 per cent solutions. They then tested acid sodium salts of the four fractions. As these were fairly soluble and much less toxic and less irritating than the ethyl esters, they were used for the treatment of tuberculous guinea pigs. They were found tuberculocidal in dilutions not higher than 1:10,000. All the untreated tuberculous pigs in the experiment died except 1 while only 1 of the treated pigs died during the time of the experiment. There were marked differences in the pathologic findings between the treated and the untreated pigs, the advantage being in favor of the treated pigs. The treated tuberculous pigs gained in weight much more than the non-treated, while the treated non-tuberculous pigs gained in weight much more than the untreated non-tuberculous pigs, a fact which was thought not only to indicate that these drugs are well absorbed and non-toxic, but also to suggest the presence of a vitamine or some element which has a marked influence on weight. These experiments seem to have been carefully controlled and are suggestive, but the numbers of animals are too small to permit us to draw decisive conclusions. Further work verifying the results is promised.

Ostromushuski,<sup>49</sup> claims some good results on tuberculous guinea pigs from the use of copper gynocardate, but there was no complete cure and the preparation had to be used with care. He claims that healthy animals may be protected from tuberculous infection by its use.

In Walker's most recent report on chaulmoogra oil and its fatty acids before the annual session of the National Tuberculosis Association in May, 1922, he described his method of determining the presence of the chaulmoogrates in tissues. He was unable to find these in the tubercles of animals treated with chaulmoogric

<sup>48</sup> Jour. Lab. and Clin. Med., 1921 (6), 415.

<sup>49</sup> Jour. Russ. Phys. Chem. Soc., 1916 (42), 335.

acid and considered this failure to penetrate the tubercle to be the cause of his negative results in all his chemotherapeutic experiments with this drug.

Biesenthal<sup>50</sup> used sodium morrhuate in the treatment of severe cases of pulmonary tuberculosis and found no effect on the bacilli or sputum, or weight of patient or fever curve. He also tested<sup>51</sup> the sodium salt of chaulmoogric acid on 10 cases of pulmonary tuberculosis with no changes in sputum or in physical findings.

We can say only with regard to these unsaturated fatty acids that much further experimentation with treatment of tuberculous animals and men must be carried on before any statement can be made concerning a beneficial action in tuberculosis. The chaulmoogric acid has apparently proved itself of value in leprosy, which makes it a possible therapeutic agent in tuberculosis, but no proof has been given as yet, and Walker's evidence as to its failure to penetrate tubercles makes it seem improbable that any such proof can be developed.

#### BILE AND BILE SALTS

Deycke and Much<sup>52</sup> showed that an emulsion of tubercle bacilli mixed with 25 per cent solution of choline and allowed to stand a short time underwent a complete solution of the fatty material of the bacilli in a few minutes and the Gram staining power disappeared completely. On the basis of this bacteriolytic action of choline on the tubercle bacilli, Mehler and Ascher<sup>53</sup> attempted some therapeutic experiments. They used "Borcholin," a cleavage product of choline and non-toxic in quite large doses but causing local irritation after subcutaneous injection; intravenous injections, however, were well borne. They claim that tubercle bacilli and also other bacteria dissolve and are killed in this solution *in vitro*. Little therapeutic effect was shown either in animals or man, but the suggestion was made that it might be valuable to combine this choline preparation with some more active agent

<sup>50</sup> Amer. Rev. Tuberc., 1920 (4), 781.

<sup>51</sup> Ibid., p. 84.

<sup>52</sup> Münch. med. Woch., 1909 (56), 1985.

<sup>53</sup> Münch. med. Woch., 1913 (60), 748.

whose power could be exerted more directly on the bacillus after the bacteriolytic action of the borcholin.

Nohring's "B<sub>4</sub>" is a derivative of bile made by adding HCl to pig or ox or any other kind of bile to set free the "B<sub>4</sub>" and then this is precipitated with NaOH.<sup>54</sup> It is a yellowish powder, very little soluble in water, but easily in HCl. It is distinguished from bile acids by the fact that it does not give the Pettenkofer reaction. It contains no nitrogen and no carbohydrate and no biliverdin test could be obtained. It represents a hitherto unknown constituent of bile. No trial of this remedy in experimental animals is reported, but it is said to produce bacteriolysis of the tubercle bacilli with a chemical binding of the toxins. No immunization results. No injurious influence was noted after subcutaneous injection except temporary irritation and infiltration. Diminution of tubercle bacilli and of fever and increase of weight were noted. B<sub>4</sub> is a choline-like substance, according to Mehler and Ascher,<sup>55</sup> who state that the lymph elements of spleen and lymph glands are destroyed by choline while connective tissues are preserved. They believe that Roentgen raying of cells really means release of choline from the lecithin of the cells and that the choline formed causes cytolysis. They used a preparation which they called enzytol which equals 10 per cent borcholin. In rats and mice cytolytic changes were noted after injecting some cubic centimeters of 2 per cent choline salt solution subcutaneously. In rabbits, they injected 0.08 gram every second day intravenously. In human tuberculous patients the subjective symptoms were improved, the sputum was lessened in amount and in the number of bacilli and the bacilli changed to chains of granules.

Arthur Mayer<sup>56</sup> used borcholin with gold potassium cyanide, and believed that the borcholin dissolved the fatty waxy sheath of the bacillus, thus facilitating the entrance of the gold salt into the bacillus. Mehler and Ascher<sup>57</sup> claimed success in healing tuberculous lesions by combining borcholin with copper treatment.

<sup>54</sup> Hirsing, Beitr. Klin. Tuberk. 1916-17 (36), 91.

<sup>55</sup> Beitr. Klin. Tuberk., 1914-15 (33), 221.

<sup>56</sup> Beitr. Klin. Tuberk., 1914 (32), 211.

<sup>57</sup> Münch. med. Woch., 1913 (60), 1041.

Takeoka<sup>58</sup> concludes from reports in the literature that the liver and the bile have certain very striking relations to bacterial infections, as follows: Posselt<sup>59</sup> states that the liver in general acts as a filter for bacteria and that the bile has a distinct inhibitory action on the growth of some microorganisms, though on the colon-typhoid group its action is favorable rather than inhibitory. Zehden<sup>60</sup> noted that tubercles were relatively infrequent in the liver and that, when they occurred, it was late in the disease, when the resistance had been broken down. Sabourin<sup>61</sup> and others noted a distinct tendency of liver tubercles to heal. This Takeoka regards as undoubtedly due to the bile. Maffacci and Sirleo<sup>62</sup> reported that tubercle bacilli injected through the umbilical cord of embryos or through the portal vein in adults were taken up by the liver cells and destroyed. Beck<sup>63</sup> noted that taurin had an inhibitory action on the growth of tubercle bacilli. On the basis of this evidence given in the literature on the effect of bile and some of its salts on the growth of tubercle bacilli *in vitro* and on the development of liver tubercles *in vivo*, Takeoka<sup>58</sup> carried on a considerable series of experiments on guinea pigs infected with human tubercle bacilli and on rabbits infected with bovine tubercle bacilli. Taurin,  $\text{NH}_2\text{CH}_2\text{CH}_2\text{SO}_2\text{OH}$ , is the most characteristic amino acid of the bile. It was first made from ox-bile and, as the yield was small, later from the muscle of a shellfish, abalone, from the juices of which it could be crystallized out in a relatively pure form. The dosage given was from 0.05 to 0.1 gram.

His results are summarized as follows: In nearly every instance the untreated controls all died before any of the treated animals. The controls lost weight almost uniformly while the treated animals gained in weight. The contrast in the extent of visible tuberculosis between controls and treated animals was marked. Whereas the process was advanced in the controls, it was arrested and in some instances apparently cured in the treated animals.

<sup>58</sup> Jour. Infect. Dis., 1917 (20), 442.

<sup>59</sup> Ergebni. allg. Pathol., 1915 (17), 719.

<sup>60</sup> Centr. allg. Path., 1897 (8), 468.

<sup>61</sup> Arch. physiol. norm. et path., 1883 (15), 52.

<sup>62</sup> Centr. allg. Path., 1895 (6), 305.

<sup>63</sup> Zeit. f. Hyg., 1894 (18), 128.

The success of the treatment varied in accordance with variations in the infecting dose, the route of inoculation and the time of beginning treatment. In all experiments, however, and in nearly every instance, the results were distinct and in many instances startling. The progress of the disease was arrested and apparently cured when treatment was begun as late as three weeks after infection. Microscopic examination of treated animals showed arrested tubercles, absence of caseation, disappearance of tubercle bacilli and evidence of repair by connective tissue ingrowth. In some treated cases the tissues were essentially normal microscopically. In treated animals, tubercles in lungs, as well as spleen and liver, are described as discrete and well localized instead of diffuse as in controls. Cavities with indurated walls are sometimes described. However, no further experiments verifying these results have been reported since this work was published. Also I have found very little inhibitory effect on the growth of human tubercle bacilli resulting from the addition of liver extract to nutrient media. Takeoka's results need further verification before we can accept the conclusion that bile or bile salts have a marked therapeutic value in experimental tuberculosis. Certainly in human pathology we often find the liver extensively invaded with tuberculous lesions in the form of a pericholangitis, characterized by actively progressing tuberculous areas in the form of cysts filled and stained with bile. Here there is no evidence that bile inhibits tuberculosis, and in guinea pigs the liver is, next to the spleen and lungs one of the most vulnerable tissues in the body.

#### CINNAMIC ACID AND THE CINNAMATES

As early as 1893, Landerer<sup>64</sup> used cinnamic acid in experimental tuberculosis in animals, claiming that it caused a marked increase in the number of white blood cells around the tubercles. Jacobson<sup>65</sup> inoculated guinea pigs with tuberculosis, treating some of the animals with ethyl cinnamate, and leaving the others untreated, testing both sets with tuberculin. The thermal reaction time was markedly shortened by the ethyl cinnamate. An

<sup>64</sup> Deut. med. Woch., 1893 (19), 204 and 228.

<sup>65</sup> Bull. Mem. Soc. Méd. Hôp. de Paris, 1919 (35), 322.

increase of leucocytes was also noted in pigs injected with ethyl cinnamate.

Corper<sup>66</sup> and others found that repurified sodium cinnamate in concentrations up to 2 per cent has no tuberculocidal action within three days, but is distinctly inhibitory to human tubercle bacilli in 5 per cent glycerine agar in a concentration of 0.05 per cent, while in rabbit blood medium, both fresh and inspissated, it is inhibitory only in a concentration of about 0.2 per cent. By continuous intravenous injection of a 2.5 per cent solution of pure sodium cinnamate, a concentration of from 0.08 to 0.16 per cent of the blood can be maintained for a period of eighty-three hours in the rabbit. On account of its hemolytic action, when administered for a prolonged period (exceeding three days), sodium cinnamate cannot be used as an inhibiting agent in tuberculosis. In concentration from 1 to 9 per cent, it gels the blood and affects the hemoglobin, the color changing to chocolate brown. In still weaker concentrations, 0.5 to 1 per cent, it produces a distinct hemolysis. Because of this toxic action on the blood, it does not seem feasible to use this preparation for the treatment of tuberculosis.

#### HYDROCARBONS

Since hydrocarbons have acquired some reputation in the clinical treatment of tuberculosis, it may be of interest to note the single experiment with these found in the literature. Volperino<sup>67</sup> treated 6 guinea pigs which had been inoculated with tuberculosis, by injection of xylene (xylol) in doses of 0.5 to 1 cc. of 10 per cent solution in olive oil. One of the 6 treated animals died on the twenty-fifth day with no sign of tuberculosis except one small nodule in the peritoneum. The other pigs were killed forty-six days after infection and compared with the controls. He found much less marked lesions than in the controls. The controls showed visible external and very widespread internal lesions by the fifteenth to eighteenth day after infection, while xylene injections begun eight to ten days after infection kept the animals

<sup>66</sup> Amer. Review Tuberc., 1920 (4), 461.

<sup>67</sup> Ann. Inst. Pasteur, 1919 (33), 191.

alive for forty days without manifestations of more than local, limited signs of infection.

Other hydrocarbons—toluene, cumene, etc.—were similarly tested with similar results. Intramuscular injections of xylene and cumene were also used in human subjects with supposed favorable results.

## CHAPTER XV

### SPECIFIC CHEMOTHERAPY WITH INORGANIC COMPOUNDS

#### METALS

Very little experimental work has been done with most of the metals that have been used in tuberculosis therapy. Lumière and Chevrotier<sup>1</sup> tested the inhibitory action on the tubercle bacillus of the salts of some thirty metals. They found that  $CdCl_3$  and  $HgCl_2$  inhibited at a dilution of 1 in 4000, while all the others, with the exception of gold, required from 2 to 20 parts per thousand.

DeWitt and Sherman<sup>2</sup> found that it required 5 per cent of  $CuCl_2$  to kill human tubercle bacilli in twenty-four hours, 1 in 100,000 of  $HgCl_2$ , 1 in 20,000 of  $AuCl_3$ , and 1 in 10,000 of  $AgNO_3$ . Silver has been tried as a chemotherapeutic agent when combined with trypan blue, but it showed no curative action.

Maurice Smith<sup>3</sup> tested the effect of a double salt of methylene blue and silver nitrate on inhibition of growth of human tubercle bacilli on broth cultures and also as a chemotherapeutic agent in experimental tuberculosis in guinea pigs. He found complete inhibition by a dilution of 1 in 100,000 but its chemotherapeutic action was negligible.

Only the following four metals—arsenic, copper, gold and mercury—have been subjected to thorough systematic investigation regarding their chemotherapeutic efficiency in experimental tuberculosis.

#### ARSENIC

Arsenic compounds have been used for many years in medicine and it is no wonder that they were soon used for the treatment of

<sup>1</sup> Bull. gén. Therap., 1913 (165), 959.

<sup>2</sup> Jour. Infect. Dis., 1914 (15), 245.

<sup>3</sup> Amer. Review Tuberc., 1922 (6), 183.

tuberculosis. In 1883, Buchner<sup>4</sup> stated that bacilli cannot be killed directly in the body and that one should treat infectious diseases with drugs which increase the resistance of the tissues against the bacilli. "This can be done only by exciting inflammatory changes of the tissues, for this is the natural reaction of the body leading to healing." Such dynamogenic influence he ascribed especially to arsenic, antimony and phosphorus. He claimed that these, in very small doses, could excite inflammation without harm. He considered that arsenic could be used both as a prophylactic and as a curative agent, and gave arsenic acid in doses of 2 to 10 mgm. daily. Weismayr<sup>5</sup> says that the antiseptic influence of arsenic is inconsiderable. Buchner himself asserts that the tubercle bacilli are not killed, but the resisting power of the tissues is increased, for, as has been said, "arsenic is the digitalis of metabolism."

Under the influence of arsenic acid, yeast loses the power to ferment sugar and growth is checked. Unorganized ferments are not influenced. Weismayr credits Robin and Binet as stating that one of the most important phenomena of phthisis is the increased fixation of oxygen and production of CO<sub>2</sub> on the part of the organism and the whole therapeutic effort must be to modify this chemism and bring it as near as possible to normal relations; they say that cod liver oil and arsenic are drugs which work in this way. Weismayr also refers to Pokhorow's view that the use of arsenic in tuberculosis is tonic, improving the general condition, increasing appetite and weight. Cacodylic acid and sodium cacodylate were also recommended, but with little result. Atoxyl and salvarsan were also used, especially in cases having both syphilis and tuberculosis. Syphilis was healed and tuberculosis either unaffected or injuriously affected.

Arkin and Corper<sup>6</sup> tested the tuberculocidal power of sodium arsenite, sodium cacodylate, mercury cacodylate, atoxyl, arsacetin, and neosalvarsan and found none, except in the case of mercury cacodylate in which the bactericidal power was about what was to be expected from the content of mercury. By analysis, they

<sup>4</sup> Quoted by Weismayr in Ott's Chem. Path. der Tuberc., p. 480.

<sup>5</sup> Ott's Chem. Path. der Tuberc., 1903, p. 481.

<sup>6</sup> Jour. Infect. Dis., 1916 (18), 368.

found that after treatment of tuberculous animals with the various arsenic preparations, arsenic was demonstrable in the liver, lungs, kidneys, blood, spleen and tuberculous tissues (lymph glands of guinea pigs and eyes of rabbits), the concentrations in the different tissues not being greatly different. There was no evidence of accumulation in tuberculous tissues. These authors draw the conclusions that arsenic cannot be said to have any specific action on human tubercle bacilli and, if of value in the treatment of tuberculosis, must be so because of favorable influence on metabolism.

#### COPPER

As early as 1885, A. Luton and later his son E. Luton<sup>7</sup> published a monograph presenting the claims of copper as a specific in tuberculosis. They claimed that it caused a local and a general reaction similar to that caused by tuberculin and that it bore toward tuberculosis very much the same relation as that borne by mercury toward syphilis. Their conclusion, after use of copper phosphate or acetate in 57 cases of tuberculosis, was that in the existing state of knowledge they could say, "il y a quelque chose," a conclusion which was echoed later by von Linden and her co-workers, Meissen and Strauss. Von Linden began her reports in 1912<sup>8</sup> and has published at intervals ever since. The last communication<sup>9</sup> seen by us reiterates what she had said in other communications: "that 0.002 mgm. of copper uniformly distributed in the media was sufficient to kill 1 mgm. of tubercle bacilli; that the green coloring of the bacterial clumps on media containing 1:1,500,000 of copper was specific for tubercle bacilli as no other bacteria showed it." She regards it as due to binding of copper by the waxy sheath containing fatty acids. Also that guinea pigs treated with copper showed a more chronic course than others not so treated, while in infected guinea pigs treated with a large initial dose (3 to 5 mgm. of copper), the disease was checked and the animals did not die of tuberculosis. In severe

<sup>7</sup> Traitement de la Tuberculose par les sels de Cuivre. Paris, 1894, G. Steinheil.

<sup>8</sup> Münch. med. Woch., 1912 (59), 2560.

<sup>9</sup> Cent. f. Bakt., 1920 (85), 136.

infections of guinea pigs, 12 injections totaling 10 mgm. of copper have sufficed to accomplish healing, "although still some isolated encapsulated foci were found not yet sterile." While she used a number of preparations of copper, the one preferred was one in which lecithin was combined with copper and which was called Lecutyl. Strauss<sup>10</sup> reports on a number of cases of lupus treated by him with copper with favorable curative and cosmetic effects.

Meissen<sup>11</sup> reported his experience in the treatment of 47 moderately severe cases of pulmonary tuberculosis in which 80 per cent of good results were obtained under treatment with copper, materially better, he thinks, than in similar cases where only the usual treatment was used. Selter,<sup>12</sup> Mayer,<sup>13</sup> Pekanovich,<sup>14</sup> Kaiser,<sup>15</sup> Moewes and Jauer,<sup>16</sup> and Eggers,<sup>17</sup> some working on animals and some on human patients, all using copper in some form but mostly in the form of von Linden's own preparations, reached the conclusion that there was no or very little favorable influence of copper on tuberculosis and none that could be called specific for that disease.

Huber<sup>18</sup> found that certain compounds of copper with amino acids (leucinate, glycinate and glutaminic acid) produced the same physiologic effects as a simple inorganic salt (copper sulphate), which is probably also true of the lecithin preparation recommended by von Linden. DeWitt and Sherman<sup>19</sup> found that it required 5 per cent concentration of copper chloride to kill tubercle bacilli in twenty-four hours, even if the organisms were spread out in a thin layer and dried on garnets, while 25 per cent did not kill all in twenty-four hours if they were in clumps; 1:100,000, however, inhibited their growth. Corper<sup>20</sup> investigated carefully

<sup>10</sup> Beitr. Klin. Tuberk., 1915 (34), 107.

<sup>11</sup> Beitr. Klin. Tuberk., 1912 (23), 215.

<sup>12</sup> Beitr. Klin. Tuberk., 1912 (24), 261.

<sup>13</sup> Ibid., 1914 (32), 211.

<sup>14</sup> Deut. med. Woch., 1913 (39), 1352.

<sup>15</sup> Therap. Monatsh., 1914 (28), 748.

<sup>16</sup> Münch. med. Woch., 1914 (61), 1439.

<sup>17</sup> Beitr. Klin. Tuberk., 1921 (47), 373.

<sup>18</sup> Jour. Pharm. and Therap., 1918 (11), 303.

<sup>19</sup> Jour. Infect. Dis., 1914 (15), 245.

<sup>20</sup> Jour. Infect. Dis., 1914 (15), 518.

the effect of copper in tuberculous animals. Rabbits with tuberculous eyes were fed copper sulphate in large amounts up to a total of 1380 mgm. of copper, and copper sulphate and copper amino acids were injected intramuscularly up to 55 mgm. of copper in ninety days, but no copper was found in the tuberculous eyes, and there was no effect on the course of the disease. Tuberculous guinea pigs treated in various ways with several simple salts of copper, showed no copper in tuberculous glands or pus and there was no appreciable effect on the course of the disease. Colloidal copper also, injected intravenously into tuberculous rabbits, was found in the liver, kidneys and lungs, but not in the normal or tuberculous eyes. So far from a specificity of copper for tubercles and tuberculous tissues, no copper in any of the forms used was found in tuberculous tissues, nor did copper have any effect on the progress of the tuberculous infection in either rabbits or guinea pigs. It appears from Corper's experiments and analyses, that in whatever form copper is administered, it is distributed not in salt form but as colloidal copper, which, according to the experiments of Wells and Hedenburg<sup>21</sup> would not be expected to enter necrotic tuberculous tissues.

In 1916, Koga<sup>22</sup> announced that guinea pigs infected with 2 mgm. of a culture of human tubercle bacilli and treated with a copper compound called cyanocuprol lived longer, increased in weight, showed diminution in number of bacilli and an arrest of pathologic lesions. Koga says that it improves or apparently cures pulmonary and surgical tuberculosis in the first and second stages and seems to produce beneficial effects upon the disease in the third stage. Cyanocuprol, according to Sugai,<sup>23</sup> is a combination of two parts of potassium cyanide and one part of copper cyanourate, and occurs as small white crystals, soluble in alcohol and water. The lethal dose for dogs is 5 mgm. per kilo of body weight. The dose is 0.25 to 0.3 mgm. per kilo of body weight, injected approximately every ten days. He reported beneficial results. Sugai also states that simultaneous injections of this drug with

<sup>21</sup> Jour. Infect. Dis., 1912 (11), 349.

<sup>22</sup> Jour. Exp. Med., 1916 (24), 107, and Jour. Bacteriology, Tokio, 1916, November, No. 254.

<sup>23</sup> Jap. Med. Literature, 1916 (1), pt. 2, 3 and 4.

a large dose of a bacillary emulsion gave complete protection. The drug indefinitely prolongs the life of previously infected animals. A symposium of Japanese workers on cyanocuprol<sup>24</sup> reported results similar to those already reported, but *all agree* that this remedy is *not a specific*, but must be judiciously used with all other possible means for improving the general condition. They also caution against continuing the injections too long in cases in which improvement comes to a standstill.

Potassium cuprocyanide ( $K_3Cu(CN)_4$ ) is said to have properties similar to those of cyanocuprol, and although not strongly bactericidal it produces, like so many other substances, an inflammatory reaction about tuberculous lesions in guinea pigs.<sup>25</sup>

Ellis<sup>26</sup> recommended for treatment of lupus and cutaneous tuberculosis a picric-brass paste composed of the basic salts of copper and zinc, which causes destruction of the tuberculous tissue with hyperemia of neighboring tissues. The treatment usually does not need to be repeated more than three or four times.

While copper in its many salts and compounds has repeatedly been shown to have no specific tuberculocidal effect and no specific therapeutic effect on experimental tuberculosis in guinea pigs and rabbits, new investigators or old investigators with new compounds are constantly coming forward with new recommendations, quite as if copper therapy had never been heard of. In all probability, so far as copper is concerned, it acts alike in all compounds and preparations, and any different action is due to the other radical of the compound and not to the copper. It may, like so many other drugs, have a hyperemic or inflammation-exciting action on the tubercle which in some cases may react favorably on the tubercles, as does tuberculin. But it is more toxic and not more favorable than the tuberculin and in these cases tuberculin may better be used.

#### GOLD

The use of gold in medicine dates back at least to the eighth century, when Abu Moussa the Wise recommended it as a cure-all

<sup>24</sup> Japanese Jour. of Bacteriology, 1916, January, p. 94.

<sup>25</sup> Hollande and Gaté, Compt. Rend. Soc. Biol., 1920 (83), 178.

<sup>26</sup> Lancet, 1919, Nov. 8, p. 827.

for every known disease. Paracelsus, the alchemist and chemist, in about 1500 A.D. recommended a so-called "elixir of life" which was composed of gold and mercury, and it is to his time that the use of gold in tuberculosis is dated. On account of serious accidents it was dropped for a time until 1810, when it was revived by Chrestien whose monograph has been reviewed by White.<sup>27</sup> In 1890, Robert Koch<sup>28</sup> reported that gold cyanide completely inhibits the growth of tubercle bacilli in dilutions of 1 in 2,000,000, but that it is entirely without influence in the animal body. This fact is explained by Schumacher<sup>29</sup> by the statement that the gold cyanides quickly break down and form gold protein compounds, which soon set free colloidal-gold, while the cyanide group unites with the sulphhydril group of cystine and forms a nontoxic hydrogen rhodanide. The inhibitory action in the test tube was said to be due mainly to the cyanide and partly to free gold ions. In 1891, J. B. White recommended the use of double chloride of gold and sodium combined with iodide of manganese in the treatment of tuberculosis. This was said to cause prolongation of life in animals and in human patients, gain in weight and appetite, diminution of cough and sputum and rales, absorption of abnormal deposits and improvement of blood condition. This treatment was so promising that it was used clinically for a number of years and at the same time Gibbes and Shurley's method was being used, which combined an iodine treatment with the gold.

The next and the most extended group of experiments on the use of gold salts in tuberculosis were reported between 1912 and 1915 by a number of German investigators. Several workers described favorable results from the use of gold potassium cyanid, especially in tuberculosis of the skin. Mayer<sup>30</sup> reported good results in general tuberculosis, especially if the gold salt is combined with some substance like borcholin which is said to dissolve the fatty sheath of the tubercle bacillus. Oberstadt<sup>31</sup> reported good in-

<sup>27</sup> Am. Med. Surg. Bull., N. Y., 1894 (7), 388.

<sup>28</sup> Deut. med. Woch., 1890 (16), 757.

<sup>29</sup> Dermatol. Zeit., Berlin, 1915 (22), 10-25.

<sup>30</sup> Deut. med. Woch., 1913 (39), 1678 and Beitr. Klin. Tuber., 1914 (32), 211.

<sup>31</sup> Internat. zent. f. d. ges. Tuber. Forsch., 1917 (40), No. 1; abst. in Amer. Review Tuber., 1918 (1), 737.

fluence on the skin lesions, but no case of perfect recovery. He thinks the effect is produced more rapidly if tuberculin treatment is combined with the treatment with the gold salt. Bruck and Glück,<sup>32</sup> after a series of rather unsuccessful experiments in guinea pigs, used gold potassium cyanid in the treatment of lupus. They ascribed their failure in guinea pigs to the fact that the gold salt needs to be injected intravenously at frequent intervals and frequent intravenous injections are impracticable in guinea pigs. Bettmann<sup>33</sup> obtained similar results in the same type of cases. Junker<sup>34</sup> reports good effects from the use of gold salts in pulmonary as well as in skin tuberculosis in patients. Rosenthal<sup>35</sup> injected gold tricyanid directly into the diseased area in men, claiming good results. Poor and Geber<sup>36</sup> also report success from the use of gold potassium cyanid in patients having skin tuberculosis. A series of seven or more papers, published by Spiess and Feldt,<sup>37</sup> together or separately, between 1912 and 1916, form the most extensive contribution to the literature on the treatment of tuberculosis by means of different salts of gold. These authors consider gold cyanid and gold potassium cyanid too toxic for use in human patients. As cantharidin had earlier been found to have a marked affinity for tuberculous tissues, they conceived the idea of reducing the toxicity of the cantharidin and using it as a carrier for the specific gold cyanid. They were able to reduce the toxicity of the cantharidin 700 times without altering its affinity for tubercles by condensing it with ethylenediamine. This compound was quite nontoxic and combined readily with gold cyanid. They used first the auric salts but found them 100 to 1000 times less efficacious than the aurous salt and therefore in all their later work they used mono-cantharidyl-ethylenediamine-aurous-cyanid under the name "Aurocantan," the formula of which they give as follows:

<sup>32</sup> Münch. med. Woch., 1913 (60), 57.

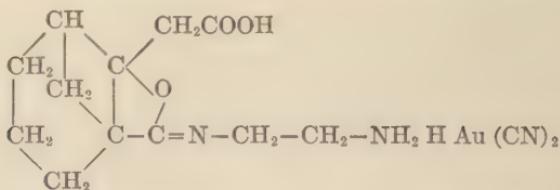
<sup>33</sup> Münch. med. Woch., 1914 (50), 798.

<sup>34</sup> Münch. med. Woch., 1913 (60), 1376; Beitr. Klin. Tuberk., 1914 (7), 421.

<sup>35</sup> Bull. gén. de Therap., Paris, 1913 (165), 804.

<sup>36</sup> Deut. med. Woch., 1913 (39), 1303.

<sup>37</sup> Deut. med. Woch., 1914 (40), 579; Berl. klin. Woch., 1915 (52), 365; Münch. med. Woch., 1914 (61), 842; 1914 (61), 1455.



The compound was first tested on about 100 animals during one and one-half years and was then used on human patients. They claim that its effects are due partly to a direct inhibitory action on the tubercle bacilli and partly to an indirect action on the defensive agencies of the body. It was believed to stimulate connective tissue formation and destroy disease products by its oxidizing power. In experimental tuberculosis in animals, they claim that weight was increased and life was prolonged and tubercles were replaced by scar tissue, especially in rabbits. In guinea pigs the effects were not so marked, a fact which they attribute to the necessity of administering the drug subcutaneously. Long continued subcutaneous treatment causes tissue necrosis, and the development of gold tolerance by the bacilli. This was shown *in vitro*, as after one and one-half months, the bacilli grew on mediums containing 1 part of gold cyanid to 400,000 parts of agar, whereas at first they would not develop in a dilution of 1 part in 2,000,000.

More recently, as an advance on aurocantan, Feldt<sup>38</sup> has recommended another gold salt, the sodium salt of p-amino-o-aurophenolcarboxylic acid, under the name of "Krysolgan" which exhibits a marked tuberculin-like effect on tuberculous lesions.<sup>39</sup> This author does not support Heubner's theory that gold owes its effect to a toxic action on the capillary endothelium, but believes that it acts as a catalyst which stimulates autolysis in the tubercles; any effect on the tubercle bacilli is secondary to this action on the tubercle.

In contrast to these favorable reports, is Koch's statement that gold salts had no beneficial effects in tuberculous animals. A number of other investigators found no or much slighter good

<sup>38</sup> Berl. klin. Woch., 1917 (54), 1111; Münch. med. Woch., 1920 (67), 1500; Therap. Monatsh., 1918 (32), 241.

<sup>39</sup> See discussion by Levy, Beitr. Klin. Tuberk., 1922 (51), 171.

effects than those that have been quoted, while Pekanovich<sup>40</sup> and Hauck<sup>41</sup> call attention to the dangers attending its use, such as anemia and icterus from destruction of blood cells and severe hemorrhages. Gelpé<sup>42</sup> notes the toxic effect of gold salts upon the capillaries of cats and dogs. He found that within a few minutes after injection, there was hyperemia and hemorrhage into the pulmonary alveoli.

DeWitt, Cadwell and Leavell<sup>43</sup> investigated the therapeutic value of various gold salts in experimental tuberculosis in guinea pigs. The treatment was given by feeding, by subcutaneous injections and by intracardiac injections. They also determined the distribution of the gold in the normal and tuberculous tissues by analysis of the tissues, as shown in the table on page 396.

DeWitt and Sherman<sup>2</sup> had found that 1 part in 1000 of gold chloride is required to kill the tubercle bacilli, while 1 per cent solutions of gold tricyanide were not always able to kill all the organisms. However, they found, as did Koch, that the gold cyanide completely inhibited growth of the tubercle bacillus in the test tube in a dilution of 1 in 2,000,000. Feldt states that these organisms develop a tolerance *in vitro* to the gold salts, while Shiga<sup>44</sup> reported that the tubercle bacilli developed no tolerance to gold salts. In our experiments, tolerance developed so slowly as not to be an important factor in the treatment of the disease in guinea pigs with gold. The stimulation of growth of tubercle bacilli by solutions of gold too dilute to kill or inhibit is an important consideration in treatment, since it is difficult to keep the concentration of gold in the animal body at or above the point required for inhibition. Both the simple gold cyanides and the cantharidylethylenediamine aurous cyanide recommended by Spiess and Feldt were used and no or very little effect was seen on the disease, except that life in general was shorter and the disease more pronounced in the treated animals than in the controls.

<sup>40</sup> Internat. Centralbl. f. d. ges. Tuberk. 1913-14 (8), 337.

<sup>41</sup> Münch. med. Woch., 1913 (60), 1824.

<sup>42</sup> Arch. Exp. Path. u. Pharm., 1921 (80), 280.

<sup>43</sup> Jour. Infect. Dis., 1918 (23), 425; Jour. Pharm. and Exp. Ther., 1918 (11), 357; Trans. National Tuberculosis Association, 1917 (13), 257.

<sup>44</sup> Jour. Bakteriol., Tokio, July 20, 1916, No. 250.

| AVERAGE                               | CANTHARIDYL* |         | Au (CN) <sub>2</sub> |         | Au (CN) <sub>3</sub> |         | Au CN   |         |
|---------------------------------------|--------------|---------|----------------------|---------|----------------------|---------|---------|---------|
|                                       | Treated      | Control | Treated              | Control | Treated              | Control | Treated | Control |
| Days lived.....                       | 130          | 218.6   | 101.7                | 161.5   | 155.5                | 161.5   | 132     | 161.5   |
| Extent of disease, per cent.....      | 60           | 35      | 95                   | 95      | 95                   | 95      | 95      | 95      |
| Milligrams gold received.....         | 13.34        |         | 150.67               |         | 210.95               |         | 74.36   |         |
| Percentage gold excreted.....         | 40.82        |         | 17.55                |         |                      |         |         |         |
| Percentage gold in organs.....        | 9.527        |         | 16.4                 |         | 0.72                 |         | 0.465   |         |
| Percentage gold in body.....          | 26.13        |         | 59.48                |         |                      |         |         |         |
| Milligrams found in liver.....        | 0.447        |         | 0.69                 |         | 0.98                 |         | 0.265   |         |
| Milligrams found in spleen.....       | 0.127        |         | 0.109                |         | 0.083                |         | 0.045   |         |
| Milligrams found in lungs.....        | 0.071        |         | 0.168                |         | 0.105                |         | 0.12    |         |
| Milligrams found in kidneys.....      | 0.12         |         | 0.06                 |         | 0.09                 |         | 0.03    |         |
| Milligrams found in lymph glands..... | 0.053        |         |                      |         | 0.02                 |         | 0.09    |         |
| Milligrams found in skin.....         | 0.45         |         | 0.27                 |         |                      |         |         |         |
| Milligrams found in intestines.....   | 0.26         |         | 0.69                 |         |                      |         |         |         |
| Milligrams found in blood.....        | 0.069        |         |                      |         |                      |         |         |         |
| Milligrams found in body.....         | 4.08         |         | 4.68                 |         |                      |         |         |         |
| Gold per gram:                        |              |         |                      |         |                      |         |         |         |
| Of liver.....                         | 0.0212       |         | 0.078                |         | 0.027                |         | 0.007   |         |
| Of spleen.....                        | 0.0916       |         | 0.1645               |         | 0.0358               |         | 0.011   |         |
| Of lungs.....                         | 0.0105       |         | 0.0305               |         | 0.009                |         | 0.0085  |         |
| Of kidneys.....                       | 0.0263       |         | 0.0087               |         | 0.0165               |         | 0.007   |         |
| Of lymph glands.....                  | 0.0246       |         |                      |         | 0.005                |         | 0.027   |         |
| Of heart.....                         | 0.009        |         |                      |         |                      |         |         |         |
| Of blood.....                         | 0.006        |         |                      |         |                      |         |         |         |
| Of skin.....                          | 0.027        |         | 0.0247               |         |                      |         |         |         |
| Of intestines.....                    | 0.0075       |         | 0.013                |         |                      |         |         |         |
| Of body.....                          | 0.012        |         | 0.0145               |         |                      |         |         |         |

\* Cantharidyl ethylenediamine aurous cyanide or "Aureocantin."

The table on page 396 summarizes these results and the amount of gold found in the different organs.

The amounts of gold found in the liver, spleen and lungs and other sites favored by tubercles are many times the concentration necessary in the test tube to inhibit growth completely. Why then do tubercles grow luxuriantly in organs containing such an amount of gold? Several possibilities present themselves. (1) The gold may be so combined with the cell proteins or be in such an inert colloidal form as to be powerless to act on the tubercle bacilli (though Feldt's experiments seem to show that colloidal gold formed with the help of asparagin has an inhibitory value equal to that of gold tricyanide). (2) The bacilli may be so protected by their membrane or by their position in cells that the gold has no power to act on them. (3) The bacilli may have acquired a tolerance to gold so that they are not affected by it. From all the experiments, we may conclude that the use of gold salts is not without danger. When active, their action seems associated with marked hyperaemia and possible hemorrhages in the neighborhood of the tubercles. This tuberculin-like reaction might, if uniform and if it could be regulated and relied upon, produce certain beneficial connective tissue changes in the neighborhood of the tubercle, but the regulation seems too difficult and dangerous to make gold therapy ever a treatment of choice.

#### MERCURY

Perhaps no drug has a longer history of use in therapy than has mercury. Of course its main specific use has been in the treatment of syphilis but it has been used in various forms and by various methods of administration to a greater or less extent in the treatment of tuberculosis since the time of Paracelsus, early in the sixteenth century. Hall,<sup>45</sup> while not regarding it as a specific, thought that "small doses of mercurial preparations are our most potent chemical weapons against this disease." Many others, at about the same period, published favorable reports on mercury therapy of tuberculosis. In 1908 and 1909, Barton L. Wright reported clinical observations with mercuric succinimide and

<sup>45</sup> Amer. Jour. Med. Sci., 1889 (98), 45.

potassium iodide alternately and his favorable reports were followed by those of many others who used the same or similar treatment. The general consensus of opinion at that time seemed to be that mercurials had a good effect on the condition of the patient but that mercury was in no sense a specific in tuberculosis.

Very little has been done until recently on mercury and mercurials in the true chemotherapeutic sense, if we may regard chemotherapy, as Lewis<sup>46</sup> says "as conveying the implication of biological experimentation carefully coördinated with constructive chemical manipulation and even, if necessary, chemical research of a most advanced type."

George Cornet<sup>47</sup> tested mercuric chloride among a series of disinfectants in 8 guinea pigs. He injected mercuric chloride for ten days until the animals began to show toxic effects and then inoculated them with tubercle bacilli. He was unable to note any difference in the extent of the disease between the mercurialized animals and the controls. Some died early of mercurial poisoning and all the others showed generalized tuberculosis.

Robert Koch<sup>48</sup> reported that mercury in vapor form inhibited the growth of the tubercle bacillus in the test tube but was entirely without influence on the progress of the disease in animals.

DeWitt<sup>49</sup> showed that mercury trypan-blue had a high bactericidal action on the tubercle bacillus and that its chemotherapeutic power in guinea pigs infected with tuberculosis was much greater than either trypan blue or the silver, iron or copper salts of that dye. The animals lived longer than the controls and showed much less extent of the disease. In view of the long continued and repeatedly favorable clinical use of mercurials in human tuberculosis and the paucity of experimental evidence as to their value, DeWitt<sup>50</sup> considered it advisable to investigate the value of mercury and its compounds in considerable detail.

Important in this connection was (1) the stability of the preparation in the animal tissue, (2) its method and form of absorp-

<sup>46</sup> Harvey Lecture, 1916, p. 113.

<sup>47</sup> Zeit. Hyg. u. Infekt., 1889 (5), 98.

<sup>48</sup> Verh. d. X. Internat. Med. Kong., 1890-91 I, Berl. klin. Woch., 1890 (27), 736.

<sup>49</sup> Jour. Infect. Dis., 1914 (14), 498.

<sup>50</sup> Jour. Infect. Dis., 1921 (28), 150.

tion and excretion, (3) the place of deposition of the mercury not excreted, and (4) the toxicity and pathogenicity of the preparations.

Moseley<sup>51</sup> states (1) that French workers claim that mercurials are converted in the stomach and intestines into mercuric chloride and circulate as the double salt of mercury and sodium; (2) that it is proved that mercury is absorbed and escapes as an albuminate in every excretion of the body, especially in the urine; (3) that a single dose is excreted within twenty-four hours; (4) that it accumulates, if given in small doses, and is deposited in all the organs. Blumenthal and Oppenheim<sup>52</sup> state that after the use of certain organic compounds of mercury a deposit of mercury was found constantly in the liver; sometimes in the intestines, and occasionally in the lungs and in the blood.

Schamberg, Kolmer and Raiziss<sup>53</sup> report that insoluble preparations injected intramuscularly are absorbed at the rate of a little over 1 per cent a day and that after six or seven weeks, almost 50 per cent of the mercury of insoluble preparations may be still unabsorbed at the site of injection. They also say that mercury has a great affinity for kidney cells and that the kidney is one of the earliest organs involved in mercurial intoxication. According to Sansum<sup>54</sup> mercuric chloride kills, on the average, in intravenous doses above 4 mgm. per kilogram of body weight. Abelin<sup>55</sup> states that the toxic influence of mercury compounds is in certain relation with their chemical structure and that the toxicity can be diminished by introduction of sulpho or sulphamino groups or through double carbon connection of mercury.

Since the bactericidal power of mercurials has long been recognized, but their use as internal antiseptics has been limited by their toxicity, the question of lowering the toxicity without lowering the bactericidal and inhibitory power is extremely important. Schrauth and Schoeller<sup>56</sup> showed that the disinfectant powers of

<sup>51</sup> Calif. State Med. Jour., 1909 (7), 338.

<sup>52</sup> Biochem. Zeit., 1914 (65), 460.

<sup>53</sup> Boston Med. and Surg. Jour., 1915 (162), 826.

<sup>54</sup> Jour. Amer. Med. Assn., 1918 (70), 824.

<sup>55</sup> Deut. med. Woch., 1912 (38), 1822.

<sup>56</sup> Zeit. f. Hyg. Infek., 1916 (82), 279.

organic mercury compounds were increased by substitution of the less acid phenolic hydroxyl for the carboxyl. They also showed that of the three isomeric mercuriated cresols, the meta derivative is the most potent disinfectant, while the ortho-hydroxy-mercuriophenoxide is more active than the para compound, and also that the entrance of a second hydroxymercuric group increases disinfectant power.

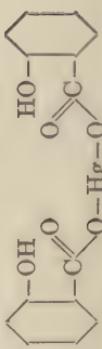
Twenty-four mercurial compounds, some inorganic and some organic were tested by DeWitt<sup>50</sup> as to their bacteriostatic and their chemotherapeutic power. The following tables indicate briefly the results.

From these tables, it may be seen that three of the mercurials used prolonged life and the animals showed a less extensive and pronounced disease than the controls and than the other animals of the series. I have continued these investigations by testing a considerable number of new organic preparations of mercury with various phenolic compounds and various aniline compounds. The bacteriostatic power of these compounds was investigated<sup>57</sup> and found often high and to vary markedly with the chemical formula and with the position of the mercury and of other groups. The power of phenol and aniline to inhibit the growth of the tubercle bacillus is greatly increased by the substitution of a mercury salt in place of one of the hydrogens, the position of the mercury altering the degree of bacteriostatic action. One NO<sub>2</sub> group in phenol or aniline, or in their mercury compounds, considerably increases the bacteriostatic action, the position of the NO<sub>2</sub> having much to do with the degree of this increase. Mercury bridge compounds, where the mercury is bound to two carbons, also have a high inhibitory power over the growth of tubercle bacilli. Methyl and ethyl groups substituted in these compounds do not greatly alter their inhibitory power. Nitroso (NO) groups have about the same or greater effect than NO<sub>2</sub>. The action of 9 mercury phenols, 14 mercury anilines and 5 other organic mercurials was reported in this paper and therapeutic results on experimentally tuberculous guinea pigs will be reported later and also results with other organic mercurials.

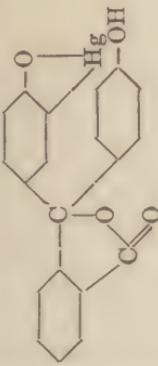
<sup>57</sup> Jour. Infect. Dis., 1922 (30), 363.

| MERCURIAL  | SOLUBLE IN                 | GROWTH IN TUBES |         |             |             |             |             | Control<br>mgm. |
|--|----------------------------|-----------------|---------|-------------|-------------|-------------|-------------|-----------------|
|  |                            | 1:1,000         | 1:5,000 | 1:10,000    | 1:20,000    | 1:50,000    | 1:100,000   |                 |
| Mercuric chloride  | Water                      | None            | None    | None        | None        | Good        | Good        | Good 0.093      |
| Mercury sulphocyanide  | Insoluble, good suspension | None            | None    | None        | None        | Good        | Good        | Good 0.031      |
| Mercury potassium cyanide  | Water                      | None            | None    | None        | None        | Good        | Good        | Good 0.034      |
| Allyl alcohol mercuric acetate   | Dilute alkali              | None            | None    | None        | None        | Very slight | Slight      | Good 0.012      |
| Mercuric succinimide   | Water                      | None            | None    | None        | None        | Good        | Good        | Good 0.025      |
| Mercuric salicylate  | Insoluble, good suspension | None            | None    | None        | None        | Good        | Good        | Good 0.021      |
| Mercurio-iodohemol   | Insoluble, poor suspension | None            | None    | None        | None        | Good        | Good        | Good 0.12       |
| Mercurol (mercuric n-ecinate)  | Water                      | None            | None    | Slight      | Good        | Good        | Good        | Good 0.02       |
| Mercury phenolphthalein  | Dilute alkali              | None            | None    | None        | Good        | Good        | Good        | Good 0.039      |
| Fluoresccin mercuric chloride  | Dilute alkali              | None            | None    | None        | Good        | Good        | Good        | Good 0.016      |
| Ortho - oxybenzylidene amino phenyl para mercuric acetate                | Dilute alkali              | None            | None    | None        | Good        | Good        | Good        | Good 0.022      |
| 1-amino-2-(p-naphthylamin azophenyl) mercuric acetate - 5-sulphonic acid | Dilute alkali              | None            | None    | None        | Very slight | Slight      | Very slight | Good 0.017      |
| Trypan blue mercuric chloride  | Slightly in hot water      | None            | None    | Good        | Good        | Good        | Good        | Good 0.03       |
| Methylene blue mercuric chloride   | Insoluble, good suspension | None            | None    | None        | Slight      | Good        | Good        | Good 0.014      |
| Iod methylene blue mercuric chloride                                     | Insoluble, good suspension | None            | None    | Very slight | Good        | Good        | Good        | Good 0.024      |

*List of compounds*

| NAME OF COMPOUND                    | PERCENT-AGE OF MERCURY | CHEMICAL FORMULA   |
|-------------------------------------|------------------------|--|
| Mercurous chloride.....             | 84.92                  | (Hg-Cl) <sub>2</sub>   |
| Mercuric chloride.....              | 93.78                  | Hg<br> <br>Cl  |
| Mercuric sulpho-cyanide.....        | 63.29                  | Hg<br> <br>S-C≡N   |
| Mercury potassium cyanide.....      | 68.72                  | Hg (CN) <sub>2</sub> 2 K C N<br>H <sub>2</sub> -C-Hg-O-C-CH <sub>3</sub>   |
| Allyl alcohol mercuric acetate..... | 63.3                   | H-C-OH<br>H <sub>2</sub> -C-OH   |
| Mercuric succinimide.....           | 50.5                   | $\left[ \begin{array}{c} \text{H} & \text{C}-\text{C}=\text{O} \\   &   \\ \text{H} & \text{H} \\   &   \\ \text{H} & \text{C}-\text{C}=\text{O} \end{array} \right]_2$ Hg |
| Mercuric salicylate.....            | 42.2                   |   |

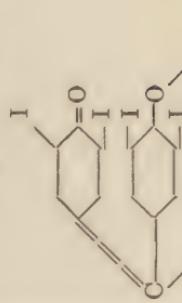
|                                  |      |   |
|----------------------------------|------|---|
| Mercurio-iodo hemol (Merck)..... | 12.0 | Patented Preparation of Hemoglobin, Mercury and Iodin |
| Mercurol (Merck).....            | 10.0 | Patented Preparation of Mercury and Nucleinic Acid    |



I

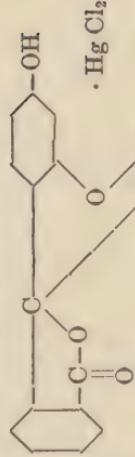
Mercury phenolphthalein.....

39.0



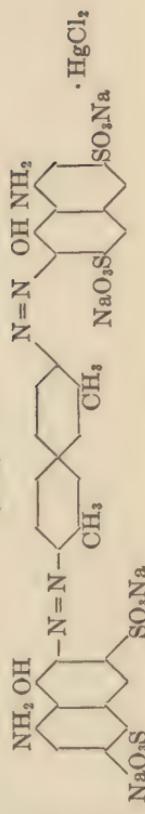
Mercury tetraiodophenolphthalein.....

19.6



Fluorescein mercuric chloride.....

33.17



Trypan blue mercuric chloride.....

15.65

*List of compounds*

| NAME OF COMPOUND  | PERCENT-AGE OF MERCURY | CHEMICAL FORMULA |
|---|------------------------|------------------|
| Ortho-oxy-benzylidene amino phenyl para mercuric acetate.....                 | 44.0                   |                  |
| 1-amino-2-[para-naphthalim-azophenyl] mercuric acetate 5-sulphonic acid ..... | 34.2                   |                  |
| Methylene blue-mercuric chloride ..   | 28.94                  |                  |
| Iod-methylene blue mercuric chloride .....                                    | 24.75                  |                  |
| Methylene green mercuric chloride ..  | 31.6                   |                  |

|  |      |  |
|--|------|--|
| Diazo-amino methylene blue ortho-toluidin di mercuric chloride.....          | 29.7 |  |
| Diazo-amino methylene blue bromhydrate ortho-toluidin mercuric bromide ..... | 39.5 |  |
| Diazo-amino methylene blue orthophenol mercuric chloride.....                | 29.7 |  |

*Chemotherapeutic experiments*

| MERCURY COMPOUNDS  | STRAIN<br>OF TU-<br>BERCLE<br>BACILLI | LENGTH OF LIFE<br>COMPARED WITH<br>CONTROLS | EXTENT OF DISEASE<br>COMPARED WITH<br>CONTROLS |
|--|---------------------------------------|---|--|
| Mercurous chloride.....  | O. H.                                 | Greater                                     | Same   |
| Mercury sulpho cyanide.....  | O. H.                                 | Slightly less                               | Less   |
| Mercury potassium cyanide.....   | O. H.                                 | Sl. greater                                 | Same   |
| Allyl alcohol mercuric acetate.....  | 1305                                  | Same  | Same   |
| Mercuric succinimide.....  | O. H.                                 | Greater                                     | Less   |
| Mercuric salicylate.....   | M.                                    | Less  | Same   |
| Merculo-iodo-hemol.....  | O. H.                                 | Slightly less                               | Same   |
| Mercurol (Merck).....  | O. H.                                 | Less  | Slightly less                                  |
| Mercury phenolphthalein.....   | O. H.                                 | Greater                                     | Same   |
| Mercury tetraiod phenolphthalein   | O. H.                                 | Same  | Same   |
| Fluorescein and mercuric chloride  | O. H.                                 | Less  | Same   |
| Trypan blue mercurous chloride..   | O. H.                                 | Same  | Same   |
| Trypan blue mercuric chloride ...  | O. H.                                 | Same  | Same   |
| 4-ortho-oxybenzylidene amino<br>phenyl para mercuric acetate...                    | O. H.                                 | Less  | Same   |
| 1-amino-2 (p-naphthalin azo<br>phenyl mercuric acetate) 5-sul-<br>phonic acid..... | O. H.                                 | Less  | Less   |
| Methylene blue and mercuric<br>chloride.....                                       | O. H.                                 | Much greater                                | Much less                                      |
|  | 1305                                  | Same  | Same or less                                   |
| Iod methylene blue and mercuric<br>chloride.....                                   | O. H.                                 | Same  | Same   |
| Methylene green and mercuric<br>chloride.....                                      | O. H.                                 | Greater                                     | Less   |
| Diazo-amino methylene blue<br>o-toluidin mercuric acetate.....                     | 1305                                  | Same  | Same   |
| Diazo amino methylene blue brom<br>hydrate o-toluidin mercuric<br>acetate.....     | 1305                                  | Same  | Same   |
| Diazo amino methylene blue<br>o-phenol mercuric chloride ....                      | 1305                                  | Greater                                     | Less   |

As far as the results have been reported, no mercurials have cured tuberculosis in any guinea pig, but some of the results have seemed somewhat more favorable than have resulted from any other metal or from any other compound so far investigated in this laboratory. This seems to make the hope possible that some compound of mercury may yet be developed which may com-

bine low toxicity with high bactericidal or bacteriostatic power and with low organotropism and thus have high chemotherapeutic power in tuberculosis.

#### COLLOIDAL METALS

P. Courmont and A. Dufornet<sup>58</sup> reported a series of experiments on the effect of various colloidal metals on the growth of Koch's bacillus on fluid media. They found pure ascitic fluid better than broth as it did not cause precipitation of the colloidal metals. Platinum, gold, silver, copper, palladium, rhodium and selenium were tested; 3, 6, 9, 12 and 15 drops of a colloidal suspension of each metal being added to each of ten tubes of the different media. The tubes were then planted with a dose of 3 drops of a suspension of tubercle bacilli and incubated. Palladium, platinum and silver showed no inhibiting action at any concentration. Copper, and rhodium showed slight inhibition in tubes containing 25 drops of the colloidal metal while selenium and gold arrested development in doses of 3 drops per cubic centimeter without destroying vitality.

#### IODIN AND IODIN PREPARATIONS

Nolen<sup>59</sup> has reviewed the use of iodin and iodin preparations in tuberculosis and that work may well serve as the basis of discussion on this subject up to that date (1914). He says: "Iodin and potassium and sodium iodides have been used for years and recommended as specifics against tuberculosis and with just as little justice as the other so-called specifics." He recalls that DeMussy and Graucher gave, in cases of pulmonary tuberculosis, 20 drops per day of tincture of iodin in wine with sugar, with good effects. See and Sticker, according to Nolen, claimed that potassium iodide caused inflammation with serous exudate around the tubercles similar to the reaction to tuberculin. Hotz<sup>60</sup> suggested that iodin produces a mass reaction on tuberculous tissue. After intramuscular injection of iodoform he found signs of a transient, more acute process in the tuberculous lesion, such as

<sup>58</sup> Compt. Rend. Soc. Biol., 1913 (75), 454.

<sup>59</sup> Brauer, Schroeder and Blumenfeld's Handbuch der Tuberkulose, 1914.

<sup>60</sup> Mitt. Grenz. Med. u. Chir., 1912 (25), 100.

swelling, increased pain, redness, exudation, etc. These exacerbations lasted but a few days and were often followed by improvement with healthy granulation tissue wall and later scar formation. He regards this as justification for the term "specific influence of iodin on tuberculosis," and attributes this favorable influence of iodin on tuberculosis to the very marked lymphocytosis which regularly follows the administration of iodin. He also showed that lymphoid tissue, and especially the lymph glands themselves, develop marked antibacterial power. He decides from this and the work of Bergel<sup>61</sup> that body tissues which are rich in lymphocytes or their degeneration products have a specific digestive power on fats, waxes, neutral butter fat, etc. Bergel thus suggested a lipolytic function for lymphocytes. According to this theory, which we have been unable to verify, the marked lymphocytosis produced by iodin may explain the favorable action of iodin on tuberculosis.

Jobling<sup>62</sup> has suggested that the influence of iodin may depend on its saturation of unsaturated fatty acids present in tubercles, which otherwise inhibit proteolysis of the dead tissues that protect the bacilli from the blood and living tissues.

Aside from specific influence, it may be considered as established that iodin preparations facilitate expectoration by increasing the bronchial secretion.

Instead of salts of iodin, Winternitz recommended iodipin, an addition product of iodin and sesame oil. It has no bad taste or odor, is not absorbed until it reaches the intestine and does not easily cause iodism.

Iodoform is one of the iodin preparations most warmly recommended by many of the earlier workers, although others state that it has no action on pulmonary phthisis. Novoiodin was also recommended by Polland<sup>63</sup> in place of iodoform; it acts by cleavage of iodin and formalin and is non-toxic.

For tuberculosis of the pleura, iodin tincture, iodvasogen and vaso liniment and iod soaps are recommended by Nolen. Other iodin preparations that have been used in tuberculosis are arso-

<sup>61</sup> Münch. med. Woch., 1909 (56), 64 and 1910 (57), 1683.

<sup>62</sup> Zeit. f. Tuberk., 1914 (22), 521.

<sup>63</sup> Münch. med. Woch., 1910 (57), 1693.

iodin, combining iodin with arsenic acid; proiodin, a milk albumin iodin preparation; iodostarin, containing 47.5 per cent of iodin with tarinic acid; and dioradin, a radio-active iod-menthol. This last has been especially recommended and advertised by S. Bernheim and L. Dieuport<sup>64</sup> who claimed astonishingly good results in pulmonary tuberculosis especially in the first and second stages. Although this treatment was published in 1911 and 1912, later work seems not to have verified it, since it is rarely mentioned in later publications. Pekanovich<sup>65</sup> stated that dioradin and other iodin compounds proved of no avail in human pulmonary tuberculosis.

J. Ritter<sup>66</sup> advises tincture of iodin in milk, 1 drop each meal the first day, and increasing by 1 drop each successive day until the limit of tolerance is reached. He testifies to the efficacy of this treatment in bone and joint tuberculosis, pulmonary tuberculosis, pleurisy with effusion, gland tuberculosis, etc. He claims that it reduces the effect of secondary invaders, causes degeneration of tubercle bacilli and an increase of lymphocytes. He concludes: Iodin can be safely administered over long periods of time; is innocuous and non irritating if given in milk; it should be given in large doses to saturate the tissues and inhibit the growth of the bacilli. Increased lymphocytosis is produced by iodin therapy and this is supposed to increase the lipolytic ferment which is said to act on the fats and waxes of the tubercle bacillus.

Escobar<sup>67</sup> uses tincture of iodin as an intrapleural therapeutic agent on account of its bactericidal action, its diffusibility, absorption and rapid elimination and the way in which it modifies the soil. Croften<sup>68</sup> has used iodoform intramuscularly, intravenously and by mouth. He thinks that one of its chief actions is in converting toxin into toxoid which is not poisonous but acts as an excellent stimulus and aids in the formation of antitoxin. He gives tuberculin combined with iodoform, which reduces the

<sup>64</sup> Zeit. f. Tuberk., 1912 (19), 20.

<sup>65</sup> Deut. med. Woch., 1913 (39), 1356.

<sup>66</sup> Illinois Med. Jour., 1919 (35), 285.

<sup>67</sup> Semana Med., Buenos Aires, 1919 (26), 345.

<sup>68</sup> Brit. Med. Jour., 1915 (1), 288.

reaction. Antomicci<sup>69</sup> also used iodoform in glycerine for tuberculous pleurisy and peritonitis, with excellent results.

Much more might be written regarding the clinical use of iodin and its preparations, but the biologic experimentation which represents the true chemotherapy has been only begun so far as iodin is concerned. Clinical use has been based on the ready penetration of the drug, its absorption, its bactericidal action and its power to cause lymphocytosis. Wells and Hedenburg<sup>71</sup> have exhaustively considered the question of permeation of tuberculous tissues with iodin compounds and subsequent analysis of tuberculous and non tuberculous tissues for iodin. Guinea pigs were inoculated subcutaneously with human tubercle bacilli and were selected for analysis just when the tuberculous glands were largest before they ruptured and discharged. In order to have similar organs or tissues to compare, human tubercle bacilli were inoculated into the vitreous of one eye of rabbits, using the other normal eye, just before the tuberculous eye was ready to rupture, for comparison. The iodin injections were made subcutaneously with the following iodin compounds: Potassium iodide, 1 cc. of a 5 per cent solution (0.050 gram of KI) generally per 100 grams of animal weight. Iodoform was given in 10 per cent emulsion in olive oil, in doses of about 1 cc. per 100 grams animal weight. Iodipin, 25 per cent iodin, was given in doses of about 0.5 cc. per 100 grams. Ethyl iodid was given in doses of 1 cc. per kilo. After the expiration of the designated time, the animals were bled, the tissues removed carefully, ground, dried and kept until ready for analysis. Hunter's method of analysis was first used instead of the less accurate method of Baumann used by Oswald Loeb and Michaud<sup>70</sup> in a similar investigation; later, Kendall's method and afterwards a modification devised by Hedenburg was employed. Some of the results may be stated briefly in the following tables:

<sup>69</sup> Polyclinico, Rome, 1916 (23), Sept. 24.

<sup>70</sup> Biochem. Zeit., 1907 (8), 301.

*Iodin in caseous glands*

| NUMBER | BLOOD | LIVER | CASEOUS GLANDS | INJECTION        | TIME AFTER INJECTION   |
|--------|-------|-------|----------------|------------------|------------------------|
| 1      | (?)   | 0.068 | 0.092          | KI               | 6 hours                |
| 2      | 0.195 | (?)   | 0.160          | KI               | 48, 24 and 6 hours     |
| 3      | 0.337 | 0.109 | 0.203          | KI               | 48, 24 and 6 hours     |
| 4      | 0.408 | 0.400 | 0.481          | KI               | 78, 42, 24 and 6 hours |
| 5      | 0.550 | 0.580 | 0.790          | KI               | 48, 24 and 6 hours     |
| 6      |       | 0.100 | 0.152          | CHI <sub>3</sub> | 48 hours               |
| 7      | 0.093 |       | 0.126          | CHI <sub>3</sub> | 72, 24 hours           |
| 8      | 0.205 | 0.060 | 0.013          | CHI <sub>3</sub> | 6, 5 and 3 days        |
| 9      | 0.006 | 0.002 | 0.013          | CHI <sub>3</sub> | 5 and 3 days           |
| 10     | 0.025 | 0.065 | 0.111          | Iodipin          | 72, 36 and 12 hours    |
| 11     | 0.082 | 0.092 | 0.044          | Iodipin          | 72 and 24 hours        |
| 12     | 0.0   | 0.0   | 0.0            | Iodipin          | 4, 3 and 1 day         |

*Iodin in normal and tuberculous eyes*

| NUMBER       | WEIGHT OF EYE |        | TOTAL IODIN |        | IODIN PER GRAM |        | FORM OF IODIN INJECTED |  |
|--------------|---------------|--------|-------------|--------|----------------|--------|------------------------|--|
|              | Normal        | Tuber- | Normal      | Tuber- | Normal         | Tuber- |                        |  |
|              |               |        |             |        |                |        |                        |  |
| 7            | 2.2           | 4.5    | 0.48        | 1.7    | 0.220          | 0.381  | KI 4 hours             |  |
| 8            | 2.7           | 3.6    | 0.49        | 0.54   | 0.182          | 0.150  | KI 6 hours             |  |
| 9            | 3.2           | 4.0    | 0.25        | 0.67   | 0.078          | 0.166  | KI 8 hours             |  |
| 10           | 2.8           | 4.8    | 0.0         | 0.0    | 0.0            | 0.0    | KI 12 hours            |  |
| 15           | 4.0           | 4.8    | 0.15        | 1.38   | 0.038          | 0.268  | Iodoform               |  |
| 16           | 3.0           | 4.8    | 0.28        | 0.58   | 0.093          | 0.117  | Iodoform               |  |
| 20           | 2.0           | 5.2    | 0.015       | 0.033  | 0.0075         | 0.006  | Iodipin                |  |
| 21           | 2.2           | 5.8    | 0.062       | 0.86   | 0.028          | 0.148  | Ethyl iodid            |  |
| Average..... | 2.76          | 4.7    | 0.216       | 0.720  | 0.081          | 0.154  |                        |  |

When the caseous substance was separated from the rest of the gland substance, it contained much more iodin than did the non-caseous portion of the glands as seen in the three following experiments:

|                       |       |       |       |
|-----------------------|-------|-------|-------|
| Gland substance.....  | 0.295 | 0.285 | 0.007 |
| Caseous contents..... | 0.481 | 0.790 | 0.013 |

The conclusions from the entire investigation were as follows:

Compounds of iodin injected into tuberculous animals enter lymph gland tubercles readily so that the proportion of iodin in such tubercles is usually greater than it is in other tissues except the kidney, furthermore it is greater in the caseous contents than in the cellular peripheries of the tubercles. Tuberculous eyes usually contain more iodin than their normal mates. This property is shown not to rest on any specific character of the tubercle itself, for other necrotic tissues also take up more iodin than normal tissues. The explanation offered is that normal cells are not perfectly permeable to iodides (except perhaps kidney cells) and lose this impermeability or semi-permeability when killed or injured, thus becoming entirely permeable for crystalloids present in the surrounding fluid. As the iodin content of the blood increases and decreases with absorption and elimination, so the iodin in the necrotic area, whether tuberculous or otherwise, varies, indicating an absence of any chemical or physical binding of the iodin in such areas. A simple, inert colloid, agar jelly, implanted in the tissues, behaves in the same way. No evidence was found of any tendency for iodin compounds of whatever nature to accumulate in tubercles or other necrotic areas or to persist in such areas when disappearing from the normal tissues and the blood.

It has been shown, then, that the iodides penetrate the tubercle, according to the laws of simple diffusion of a crystalloid in solution into an inert colloidal mass, but by no means specifically and with no tendency to accumulation in the tuberculous tissue. According to the above experiments, iodin may even be found, four hours after injection, in a proportion of 38 parts per 100,000. It is important, then, to determine whether in this concentration it may exercise antiseptic or bactericidal power over the tubercle bacilli. Cantani<sup>71</sup> found that iodin in concentration of 1:500 to 1:1000 had some antiseptic action, while the concentration mentioned above would be about 1:2600, a dilution over  $2\frac{1}{2}$  times as great, and in most cases analyzed the dilution was much greater. DeWitt and Sherman<sup>2</sup> showed that Lugol's solution, which contains 1 part of iodin and 2 parts of potassium iodide to 300 parts of water, has very little bactericidal power, since in full strength solution for twenty-four hours the tubercle bacilli were not all killed. Dassy,<sup>72</sup> however, claims that the virulence of

<sup>71</sup> Zeit. f. Hyg. u. Infek., 1909 (63), 34.

<sup>72</sup> Rev. Sud.-Amer. de Endocrinologie, 1919 (2), 1.

tubercle bacilli, as well as of other bacteria, is much attenuated by growing them in an atmosphere containing iodin vapor.

Niclau and Nasba<sup>73</sup> reported that guinea pigs inoculated intraperitoneally with large doses of bovine tubercle bacilli killed by heat and three weeks later given an intraperitoneal or subcutaneous injection of 1 cc. of Lugol's solution, reacted quickly and violently and death occurred within twenty-four to forty-eight hours with complete collapse. There was intense hyperemia of all the organs. This suggestion, however, of a special reactivity of tuberculous granulation tissue, I am unable to verify, since I have injected Lugol's solution into a number of guinea pigs having advanced tuberculosis and found it no more toxic for these than for normal guinea pigs.

Cantacuzene<sup>74</sup> reported that fat-free tubercle bacilli lose much of their toxicity for guinea pigs if treated with iodin (Lugol's solution) before their inoculation and that they are then absorbed much faster than non-iodized bacilli. The resorption of fat-free bacilli and of tuberculous formations in guinea pigs, he continues, is greatly enhanced by the daily administration of potassium iodide, the iodin salt greatly stimulating the phagocytic property of the endothelial leucocytes. These observations seemed important in view of the use of iodin and iodides in tuberculosis and in conditions in which absorption of granulation tissue is desired. E. F. Hirsch<sup>75</sup> therefore repeated the experiment, inoculating a number of guinea pigs with iodized and noniodized fat-free tubercle bacilli, finding that thirty-nine days after inoculation with non-iodized fat-free tubercle bacilli there were still unabsorbed bacterial masses and granulation tissue; that even fifty-two days after inoculation with the iodized fat-free bacilli there were still unabsorbed masses. In other words, the treatment of the bacteria with the iodin solution had no apparent effect on the absorption. He also found that daily administration of potassium iodide had no apparent effect on absorption and organization of tuberculous nodules caused by fat-free tubercle bacilli. Hence Hirsch concludes that the use of iodin and iodides in facilitating the absorp-

<sup>73</sup> Compt. Rend. Soc. de Biol., 1916 (79), 541.

<sup>74</sup> Ann. Inst. Pasteur, 1905 (19), 699.

<sup>75</sup> Jour. Infect. Dis., 1914 (15), 487.

tion of necrotic material and organization of tuberculous and other granulation tissue has no experimental basis.

As to the therapy of experimental tuberculosis in animals, not much work has been published. Klemmer in 1903 stated that after injection of 25 per cent iodipin subcutaneously into guinea pigs and rabbits with tuberculosis culture, he found them to emaciate more rapidly and to die more quickly than the controls. In my own unpublished experiments, tuberculous guinea pigs were fed iodin daily, in soluble starch powder or in milk powder in amounts from 10 to 50 mgm. The treatment was begun the day after inoculation, starting with a dose of 10 mgm., increasing as rapidly as was consistent with the animal's weight, which was taken weekly. The control pigs of one set lived 42, 135 and 147 days after inoculation and all died of marked generalized tuberculosis. The treated pigs of the same set lived somewhat longer, the duration of life being 116, 137, 180, 196, 212, 379 and 459 days. All had generalized tuberculosis, although in some cases it was less marked than in the controls. In another set, which received a larger dose of virulent tuberculosis culture, the controls lived 45, 69, 84, 86, 86 and 117 days, all showing marked generalized tuberculosis, while the treated pigs lived about the same time on the average 58, 77, 78, 91, 95 and 100 days. The degree of the disease was also about the same as in the controls.

Experimental investigation of iodin as a chemotherapeutic agent, has, therefore, as yet given little proof of its value or encouragement for the hope that it may prove a real chemotherapeutic agent in tuberculosis.

#### SALTS OF CERIUM AND CERIUM EARTHS AND OTHER RARE EARTHS

Since about 1912, a number of French investigators have been testing the salts of cerium, samarium, neodymium, praseodymium and other rare metals in tuberculosis. Among these workers are Grenet and Drouin,<sup>76</sup> Rénon<sup>77</sup> and Frouin.<sup>78</sup>

Grenet and Frouin say that it has been established that the salts of cerium reduce the fats of the tubercle bacillus from 25 to

<sup>76</sup> Bull. Soc. Méd. des Hôp. Paris, 1920 (44), 589.

<sup>77</sup> Bull. Soc. Méd. des Hôp. Paris, 1920 (44), 602.

<sup>78</sup> Bull. Acad. Méd. Paris, 1920 (83), 549.

40 per cent to 22 to 16 per cent in cultures to which the salts are added, and that these salts induce mono-nucleosis both in man and animals, but some are too toxic for clinical use. Rénon adds that "the results obtained by Grenet and Drouin with the sulphates of the cerium earths are the most scientific yet realized in the chemotherapy of tuberculosis." In tuberculous patients, the expectoration became less purulent and less profuse, the physical signs were modified and disappeared and the bacilli either disappeared from the sputum or, if still found, they showed very marked modifications and took stains badly. Three guinea pigs inoculated with these modified bacilli, after losing weight for two weeks, had regained their health by the end of two months, and a fourth showed no signs of the disease, indicating a great reduction of virulence. They state that, while the action is mainly on the bacillus, the rare earths act on the organism by favoring fibrogenesis and the production of sclerosis, perhaps on account of the enormous mononuclear leucocytosis induced. They do not claim that the lesions progress to complete cure. They insist that these salts must be used only in afebrile cases of pulmonary tuberculosis. Rénon lists nearly two dozen salts of rare earths and other elements which have an inhibitory influence *in vitro* on the tubercle bacillus. Among them he includes cadmium fluoride and chloride, neodymium sulphate, and salts of bismuth, silver, gold and selenium. He suggests that chemotherapy of tuberculosis might be aided by withholding from the tuberculous those substances, such as potassium, magnesium, phosphorus, sulphur and iron which seem to be indispensable for the proliferation of bacteria in artificial culture, but does not explain how the patient is to get along without them.

Frouin reports experiments with intraperitoneal injections of sulphates of cerium earths in 50 rabbits and 30 guinea pigs after they had been inoculated with bovine tuberculosis. The final outcome confirms, he says, what might be expected considering the inhibiting influence of these salts on cultures of tubercle bacilli and the reduction of the fat and wax content of the bacilli cultivated in the presence of these salts. The animals injected developed sclerosis in the organs presenting signs of tuberculosis and, with a few exceptions, lived from two to five months longer

than the controls. In the rabbits, the tuberculous lesions remained restricted to the lungs.

The experimental observations on the cerium earth group do not warrant the amount of clinical enthusiasm that has been reported. According to the figures given the inhibitory action of these salts is not at all high, about one to five parts per thousand being required to prevent growth; this may be contrasted with gold cyanide, which inhibits when in dilution of 1:2,000,000, and yet which could not be made to show any favorable influence on tuberculosis in animals. Innumerable substances have a more powerful inhibitory effect on tubercle bacilli than these rare earth salts. Much is made of the reduction of the fat content of the tubercle bacilli grown on mediums containing these salts, the fat being reduced, it is said, from 25 to 40 per cent to 16 to 22 per cent; but we know of no evidence that such variations in fat content have any significant relation to the virulence of the tubercle bacillus for man. As far as we can learn, no attempts were made to determine the capacity of the salts of the rare earths to kill tubercle bacilli, and there is but one report made of any systematic study of their effects on experimental tuberculosis in animals, in which alone accurate controls can be made. We merely have the statement that on the basis of a slight inhibitory effect in cultures, one uncorroborated series of animal experiments, and, what is perhaps more important, a capacity to cause a lymphocytosis, a small number of cases of human tuberculosis have been treated with cerium salts by repeated intravenous injections.

#### CONCLUSIONS ON THE SPECIFIC CHEMOTHERAPY OF TUBERCULOSIS

We have discussed the subject of specific chemotherapy on the basis of the definition given by Paul Lewis, already referred to—"biologic experimentation carefully coördinated with constructive chemical manipulation and chemical research of an advanced type." This is a definition of ideal chemotherapeutic research. By no means all of the experimental work described or referred to in this section lives up to the definition in every respect. However, we have included in the section whatever work we have

found in the literature in which biologic experimentation with certain chemical substances has been used in an effort to find a chemical cure for tuberculosis. In most of the experiments described the thought has also been that of Ehrlich—the disinfection of the infected body by specific chemical agents. As has been already shown—no specific chemotherapeutic agent has been found for tuberculosis. No chemical yet used has *cured* the disease in the experimental animals employed.

Some of them have prolonged the life of the animals. Some have apparently prevented the emaciation so characteristic of the disease. Some have limited the disease, so that the treated animals showed far less advanced disease than the untreated, which were inoculated with the same dose of the same culture and had lived for the same period of time. These things are all encouraging, especially when we remember that the guinea pig, which has been used in most experiments, is much more susceptible than man and therefore much less easily cured. The aim therefore, of destroying all the tubercle bacilli in the animal body at a single dose or even after many repeated doses, has not been attained. By no means are the workers ready to give up this aim which has been pursued so long and through such devious ways. Stern<sup>79</sup> thinks that the principle of chemotherapy, in the sense of a special affinity of the bacilli or of the diseased tissues for the introduced drug, has not been proved. But he thinks there is every reason to assume that where the circulation is especially active, the drug is brought into the region; then by checking the circulation, the drug can be kept there. It is possible to influence the circulation artificially along these lines. Even the drugs which are most efficient in curing a disease, like potassium iodide in sporotrichosis or in syphilitic gummas, have no destructive action on the parasites which cause the disease. They act on the diseased tissues, perhaps by causing their disintegration, and thus influence the disease. They may have no parasiticidal power either *in vitro* or *in vivo*. It may be that in a similar way lies the future of chemotherapy in tuberculosis.

<sup>79</sup> Zeit. f. Tuberk., 1916 (26), 244.

Dale,<sup>80</sup> discussing recent tendencies in chemotherapy, states:

The rapid development of the science of immunity and its successful application in several directions to the treatment of bacterial infections, threatened to divorce pharmacology even more completely from specific therapeutics. When, however, bacteriologic methods seemed to find a limit to their efficacy and infections were encountered to which immunological treatment seemed inapplicable, the investigation of drugs and chemicals as specific remedies for infection seemed to start from a new point of view which the study of immunity had imposed. Search was to be made for new substances, produced in the laboratory, which, like nature's antibodies, should be harmful only for the infecting parasite and harmless for the host; which should have maximal "parasitotropic" and minimal "organotropic" properties. The new search for specific remedies, for "magic bullets," as Ehrlich picturesquely conceived them, which would bring down the parasite unerringly and leave the host unscathed, must be constituted as the newer science of chemotherapy which was to displace the old fashioned pharmacology, where the attack on infections by means of drugs was concerned. The problem must be the discovery of substances adapted to the receptor side chains of the protoplasm of the parasite, fixing themselves to it with a maximal affinity and bringing to bear on it the poisonous properties of a toxophore grouping and at the same time having a minimal affinity and therefore a minimal toxicity for the tissues of the host. Action on the host was to be regarded as something to be avoided and eliminated as far as possible. Its pharmacological details would be largely irrelevant. It would be foolish to deny to this newer method of attack one achievement of first class importance. The dyes, the action of which furnishes probably the clearest example of a directly "parasitotropic" action, have hardly fulfilled their earlier promises. Trypan blue seems to have established itself as a remedy for piroplasmosis in domestic animals, but beyond that, there is as yet no considerable therapeutic success to be recorded for this group. Atoxyl and later salvarsan were achieved as important results of the new chemotherapy. Tested on spirochaetes *in vitro*, however, salvarsan proved surprisingly harmless, though parasites so treated seemed to have lost their power of infection.

Levaditi found that the addition of liver emulsion gave to salvarsan the power of killing the spirochetes even *in vitro* and there are other indications that the host's tissues do not play an indifferent part in the therapeutic process. It seems possible, as suggested by Voegtlín and his co-workers in the Public Health Department at Washington, that the really effective agent is the arsenious oxide (m-amido, p-hydroxy-phenylarsenious oxide) derived from

<sup>80</sup> Proc. Royal Society of Med., 1921 (14), 7, Sec. of Ther. and Pharm.

salvarsan by partial oxidation in the body. The slow oxidation and the steady maintenance of a low concentration of the effective parasiticide is probably, according to Dale, the necessary factor in the chemotherapeutic efficiency of salvarsan, which is organotropic quite as much as parasitotropic. In the action of emetin salts in the treatment of amoebic dysentery and of quinine in malaria, Dale finds similar evidence of organotropic action combining with parasitotropic action to bring about an efficient chemotherapy. He concludes:

Brilliant with promise at its beginning, chemotherapy seems to have reached a point where its successes are the result of luck and empiric trial; nothing but fuller knowledge can give us that rational basis of theory from which an orderly, scientific progress can result.

In tuberculosis, while we shall still cling, no doubt, to the hope of finding an agent having a specific parasitotropic and bactericidal action in the body, as the simplest and most efficient mode of attack on the problem, really scientific workers will, no doubt, as they go on, study with greater intensity, the pharmacologic as well as toxic action of their chemicals on the tissues of the animal and also the action of these tissues on the chemical compound in order to attain a more thoroughly rational basis for their chemotherapeutic investigation of tuberculosis. In that way, we may hope to succeed in the future where we have failed in the past in developing an efficient chemical treatment for tuberculosis. The apparently definite chemotherapeutic effect of chaulmoogra oil and its components on leprosy gives encouragement to the hope that other acid-fast bacilli may be found vulnerable to specific chemical agents. However, we cannot emphasize too strongly the statement of Paul Lewis:

Certainly it will be a most unfortunate thing for the progress of tuberculosis research if every substance showing interesting properties in the laboratory is immediately rushed to the clinic regardless of consequences. In this situation patience is to be taken more than usually as an evidence of virtue.

## CHAPTER XVI

### THE NON-SPECIFIC CHEMICAL THERAPY OF TUBERCULOSIS

Wesener<sup>1</sup> says, "a well known axiom states that the curability of a disease is in inverse ratio to the number of drugs and therapeutic measures used against it." Thus malaria, whose treatment consists essentially of quinine and arsenic, and syphilis, with iodin, mercury and arsenic as its curative agents, are the two diseases whose drug therapy is the most satisfactory. Hence the more drugs and therapeutic measures have been and still are used against a disease, the more is the conclusion justified that we as yet know no real cure of that disease, and this is the case with tuberculosis. This has been well shown in the discussion of specific chemotherapy. It will be no less justified as we look over the field of non-specific chemical therapy. Since the development of specific vaccine and serum therapy, drug therapy has fallen somewhat into disrepute and tuberculosis treatment consists in the main of hygienic measures, palliative drugs, tuberculin and surgical procedures. However, so long as we have no satisfactory specific therapy of any kind, it will be necessary, as it has always been necessary, to use drugs to meet conditions and symptoms as they arise.

S. Solis-Cohen<sup>2</sup> says that although without proper dietetic and hygienic management, drugs are of small service, on the other hand recovery is often not complete and not maintained without them. Certain drugs have "stood the test of time" and still help tuberculous patients to recover and to stay "well." Iodin in the infiltrative stages, phenol, creosote or guaiacol in the more advanced stages or in the presence of fever, and calcium for use throughout, are perhaps the most used of these non-specific remedies in tuberculosis. These have been recommended so persistently that some experimental work has been done with them to test whether

<sup>1</sup> Die Antiparasitäre Behandlung der Lungenschwindsuche. Cent. f. Bakt., 1888 (4), 499.

<sup>2</sup> Pennsyl. Med. Jour., 1919 (23), 11.

they might be true specifics and some of them have, therefore, been discussed in the section on specific chemotherapy. Although no specific influence has been demonstrated for any of these preparations, they will still be used because of their favorable results in clinical cases.

In the following pages are discussed a few of the chemical agents that have been recommended in tuberculosis, especially those that have been subjected to some experimental investigation.

#### CALCIUM THERAPY

The features of calcium metabolism in tuberculosis have been reviewed in full in the section on metabolism (p. 295), the general conclusions being as follows: The theory that tuberculosis is accompanied by, and perhaps dependent upon a demineralization, and especially a loss of calcium from the body, has not been supported by the best controlled investigations. There is no acceptable evidence that there is any decrease in the amount of calcium in either the blood or the tissues in tuberculosis. Neither has it been shown that the amount of calcium in the blood and tissues can be measurably increased, at least more than momentarily, by feeding calcium compounds of whatever sort in tuberculosis or in any other condition in which the blood calcium is not appreciably below the normal.

Despite these facts, or in ignorance of them, there exists a widespread belief that calcium salts are of value in the treatment of tuberculosis.<sup>3</sup> This seems to rest on the following facts:

1. Calcified tubercles are, at least in man, usually healed tubercles; hence the administration of calcium should favor the calcification and therefore the healing of tuberculous lesions. (See Calcification in Tuberculosis, Chap. VI.) In support of this hypothesis we have the observation of Wersen<sup>4</sup> that children with tuberculous peribronchial glands gave fluoroscopic evidence of more rapid calcification of the glands if given calcium lactate by mouth. Also there is the reputed resistance to tuberculosis of those whose occupation causes them to inhale much lime dust. On the other hand we have no evidence that the calcium deposi-

<sup>3</sup> For full review see Leuchtenberger, Beitr. Klin. Tuberk., 1922 (50), 322.

<sup>4</sup> Upsala Läkaer. Förh., 1914 (20), 1.

tion in tuberculous lesions causes them to stop progressing; it is more probable that calcification *follows* the healing of human tuberculous lesions. In bovine tuberculosis the presence of large amounts of calcium deposit in early stages of the lesions does not seem to impede the progress of the disease. Furthermore, bone tissue itself has certainly no noteworthy resistance to tuberculous infection.

Nagai<sup>5</sup> tried the experiment of keeping guinea pigs exposed to an atmosphere in which either lime dust or lime dust plus tubercle bacilli was kept in suspension, and found no demonstrable beneficial action upon tuberculosis whether the disease was produced by inhaling lime dust plus tubercle bacilli or had its origin previous to such exposure. The lime dust was taken up by the lungs and deposited in the interstitial tissue without any apparent detrimental effects. Coutière<sup>6</sup> is among those who describes favorable clinical effects from inhalation of lime dust, but reports no experimental evidence.

2. Some early investigators thought that they had established the existence of decalcification as a characteristic and important feature of human tuberculosis. As brought out in the review of the metabolism of calcium, this hypothesis has not been established and is probably not correct. Furthermore, it is apparently not possible to modify appreciably the amount of calcium in the body by oral administration of calcium salts. Normally the blood is practically saturated with calcium to the limit of its carrying power, and therefore does not take up more calcium from the intestines. Even if excessive amounts of calcium are introduced into the blood by intravenous injection the normal concentrations are reestablished in a very short time. In view of this evidence, therefore, it seems *a priori* altogether doubtful that calcification of tuberculous lesions can be appreciably altered by oral administration of calcium salts. Whether a human tuberculous lesion undergoes calcification or not seems to depend chiefly if not solely upon how long it remains as a quiescent mass of colloidal organic material presenting such physical properties as favor the deposition of calcium salts;<sup>7</sup> i.e., a human tubercle is probably usually

<sup>5</sup> Tokio Igakukai Zasshi, 1918 (32), 2.

<sup>6</sup> Bull. Acad. Méd., 1921 (86), 410.

<sup>7</sup> Arch. Int. Med., 1911 (7), 721.

at least latent before it begins to calcify. Furthermore, tuberculous lesions in cattle show partial calcification at all stages, yet seem to progress unimpeded by this process.

It is, of course, perfectly possible that calcium is of value in the growth of connective tissue, as silica is said to be, but we lack any proof of a shortage of calcium in the tuberculous tissues, or of a greater capacity of connective tissues to grow in calcium-treated animals. Furthermore, the usual large daily intake of milk in the diet of the tuberculous patient guarantees an abundance of calcium.

3. Numerous clinical reports of favorable results from the use of calcium have been published and are entitled to consideration at least on the basis of their number. However, if there has ever been published an adequately controlled clinical study of the value of calcium therapy in tuberculosis we have not succeeded in finding it.

The French, especially, have urged the value of calcium in tuberculosis, not so much with the idea of facilitating calcification of the tubercles, as with the purpose of overcoming the supposed calcium loss in the "demineralization" of tuberculosis. A review of the literature by Max Kahn<sup>8</sup> concludes with this statement, "In looking over the mass of literature on this subject, one is left in doubt whether the use of lime in the treatment of tuberculosis is to be recommended."

Experimental evidence also seems to be lacking. Although occasionally writers on the clinical use of calcium refer vaguely to experiments which they have performed, specific reports are few. Van Gieson and Lynah<sup>9</sup> have published enthusiastic approval of Russell's<sup>10</sup> method of treatment, which was based on the doubtful theory that administration of HCl to patients increases the amount of gastric rennin which causes calcium to combine in an assimilable form with casein, and supported it only with observations on three dogs. Two of these dogs on a calcium poor diet showed more tuberculous lesions than one on a normal diet.

<sup>8</sup> Medical Record, May 23, 1914.

<sup>9</sup> New York Med. Record, 1912 (82), 883; National Tbc. Assoc., 1912 (8), 466.

<sup>10</sup> New York Med. Record, 1909 (76), 889 and 1021.

Emmerich and Loew<sup>11</sup> call attention to Hamburger's demonstration that calcium salts make the phagocytes more active, and report experiments in which the resistance of guinea pigs to anthrax seemed to be increased by calcium feeding. Mice were also made more resistant against hog cholera by feeding calcium. Their experiments on tuberculosis consisted in inoculating 22 guinea pigs with tubercle bacilli of unspecified origin or virulence. Twelve of these had been fed 0.1 gram per kilo daily of calcium chloride for from 5 to 11 months. The inoculation took place on May 18 and by October 27, 2 of the control pigs and 4 of the calcium pigs were alive, but all died before November 19. The calcium pigs in general held their weight better and showed somewhat less tendency to the presence of pulmonary lesions. As will be seen the difference obtained was only one of degree and this not constant. These authors speak also of clinical use of calcium, mentioning a favorable influence on digestion exerted by calcium chloride, but the statements as to clinical results are very vague.

Massini<sup>12</sup> injected 10 rabbits with bovine tubercle bacilli and gave 1 cc. of 1 per cent  $\text{CaCl}_2$  solution intravenously twice a week to half of them. As these treated animals lived an average of 6 days longer than the other 5 he looks upon this as a "small success," although the range in the treated animals was from 75 to 120 days, and in the untreated from 68 to 116 days. It is difficult to interpret these figures as even a "small success." Michelazzi<sup>13</sup> infected 30 rabbits and guinea pigs and found calcification of the lesions in rabbits treated with calcium; in the guinea pigs a cirrhosis of the liver in the treated animals marked an increased resistance. As the calcified lesions in the rabbits were sterile he believed that he had obtained a curative effect. This work has not, as far as we can find, been repeated and confirmed. Laitinen<sup>14</sup> reports two series of experiments (each on 20 to 30 guinea pigs) in which the animals fed calcium phosphate lived twice as long as the controls, but calcium hydroxide had no

<sup>11</sup> Arch. f. Hyg., 1913 (80), 261.

<sup>12</sup> Schweiz. med. Woch., 1921 (51), 223.

<sup>13</sup> Gaz. degli Ospedali, Milano, 1904 (25), 425; abst. Rev. de la tuberc., 1904 (1), 421.

<sup>14</sup> Ref. in Zent. f. Tuberk., 1922 (17), 317.

influence. Obviously the duration of life in so small a number of animals as this, has little significance in view of the great variations seen in any set of infected guinea pigs.

On the other hand, Leo<sup>15</sup> and Finsterwalder<sup>16</sup> observed no influence of calcium treatment on tuberculous guinea pigs. Kindborg<sup>15</sup> found that calcium salts favor growth of tubercle bacilli in culture media. This is corroborated by Tanaka who found that the calcium ion itself has *in vitro* no inhibiting action on the tubercle bacilli, but rather favors growth, although some calcium salts are inhibitory. Of these he recommends trial of calcium salicylate, which causes an increase of both red and white cells when injected intravenously in rabbits. Intravenous injections of calcium chloride into four rabbits produced in 3 a diuresis with nitrogen retention, which he thinks may be a favorable factor in tuberculosis therapy. Maver and Wells have so far failed to observe any noteworthy therapeutic effect of calcium on tuberculous guinea pigs in experiments still under way in this laboratory, and unpublished.

Another way in which calcium may possibly be of influence is through its action on the leucocytes. Hamburger found that calcium ions have a striking positively chemotactic effect on leucocytes, which has been corroborated by Miss Wolf,<sup>17</sup> but just how an increase in the calcium content of either the blood or the tissues as a whole could affect the action of leucocytes in a tuberculous lesion is not clear. Neither can we imagine any influence from calcium on the basis of the transitory rise in the proportion of polymorphonuclear leucocytes which follows injection of calcium salts (Rösler).<sup>18</sup> A general favorable effect of calcium on tissue metabolism has also been suggested.<sup>19</sup>

It must be freely admitted that when the experience of large numbers of clinicians familiar with the course of tuberculosis in man, endorses the use of calcium as giving definite clinical benefit, we must listen with respect to this endorsement, even if we cannot

<sup>15</sup> Berl. klin. Woch., 1922 (48), 1800.

<sup>16</sup> Pflüger's Arch., 1913 (153), 546.

<sup>17</sup> Wolf, Jour. Exp. Med., 1921 (34), 375.

<sup>18</sup> Wien. Arch. inn. Med., 1921 (2), 281.

<sup>19</sup> See Starkenstein, Therap. Halbmonatsch., 1921 (35), 553.

explain such results or reproduce them in experimental animals.<sup>20</sup> The resistance of workers in lime dust to tuberculosis is a worldwide belief, and hence in all probability not groundless. On the other hand the fact that most standard works on tuberculosis say little or nothing concerning calcium as an agent of value in the treatment of this disease, would indicate that the enthusiastic opinions of the few have not been corroborated by the experience of the many.<sup>21</sup> Neither are negative clinical results lacking,<sup>22</sup> and the statistical study of the influence of hardness of drinking water by Opitz<sup>22a</sup> did not disclose any more favorable tuberculosis rates in populations drinking water containing much lime.

#### SILICA THERAPY

Silicates have been considered by a few clinicians and pharmacologists to exert a favorable influence in pulmonary tuberculosis, but on two entirely distinct grounds. One is based on the more or less popular belief that persons who work at trades leading to the inhalation of certain silicious dusts are relatively free from pulmonary tuberculosis, presumably because the resulting pulmonary fibrosis helps to circumscribe and heal any existing tuberculous lesion.<sup>23</sup> (The main features of silicate deposition in the lung, including its effect on tuberculous infection, will be found discussed under Pneumonokoniosis, Chap. VI.)

An entirely different influence of silica in tuberculosis, namely, stimulation of fibrosis, has been described by several German

<sup>20</sup> See review by Tweddell, Med. Record, 1922 (101), 141.

<sup>21</sup> The use of calcium to check diarrhea, as recommended by Maendl (Zeit. f. Tuberk., 1917 (28), 334; also Med. Klin., 1920 (16), 228; Ringer and Minor, Amer. Review Tuberc., 1922 (5), 876), and others, is not based on any action on the tubercle bacillus itself, or apparently on the tuberculous lesions, but depends on the effect of calcium on exudative processes, and is analogous to the use of calcium to increase coagulation in hemoptysis. (Maendl, Zeit. f. Tuberk., 1921 (35), 184). This form of calcium therapy is not related to the use of calcium for a definite effect on the tuberculosis, and is outside the theme of our discussion.

<sup>22</sup> See, for example, Petheö (Ref. in Zent. f. Tuberk., 1922 (17), 317), who observed no benefit in 240 cases of juvenile tuberculosis.

<sup>22a</sup> Deut. med. Woch., 1920 (46), 1391.

<sup>23</sup> See Volbrath, Beitr. Klin. Tuberk., 1921 (47), 237.

investigators, inspired largely by the pharmacologist, Kober.<sup>24</sup> Despite its insolubility, small amounts of silica are absorbed, chiefly from plant foods, so that small traces are found in all the tissues and in the circulating blood. Apparently connective tissue is especially rich in silica, and some authors ascribe to this element an important function in determining the elasticity and tensile strength of fibrous tissues, an idea which may well be doubted in view of the very small amount of silica that is present. Of the viscera, especially large amounts have been described in the pancreas, which some have looked upon as the chief depository of silica for the body needs, analogous to the iodin storage of the thyroid.

Also in 1901 Hugo Schulz<sup>25</sup> took up the same topic, and himself confirmed the statement that connective tissue is comparatively rich in silica. He comments on the fact that silica seems related to the supportive structures in all forms of life, and suggests that this element may have therapeutic value in diseases in which the connective tissues are concerned. He found from 0.0614 to 0.2592 gram SiO<sub>2</sub> excreted in the urine daily, the largest amounts when the diet consisted chiefly of beans and bran bread.

Presumably the chief source of silica in the animal economy is from vegetable foods which contain much silica, and it is an interesting fact that numerous popular herb teas contain considerable soluble silica, and the same is true of certain mineral waters, some of which have had a reputation as of value in the treatment of phthisis. Zickgraf<sup>26</sup> ascribes to the silica content of a certain water an improvement in the character of the leucocytes in tuberculosis as indicated by the Arneth formula.

More recently further contributions on the same subject have appeared in Germany. Kahle<sup>27</sup> undertook in the pathological laboratory of Professor Rössle, at Jena, an investigation of the effect on experimental tuberculosis of an organic silica preparation devised by Weyland of the same university, the chemical nature of which is not disclosed. He found that in three cases of phthisis

<sup>24</sup> One of his pupils, Siegfried (Arch. de Pharmacodynamie., 1901 (9), 225) has reviewed the literature on silica up to 1901.

<sup>25</sup> Pflüger's Arch., 1901 (84), 67; Münch. med. Woch., 1902 (49), 440.

<sup>26</sup> Zent. inn. Med., 1908 (29), 509.

<sup>27</sup> Münch. med. Woch., 1914 (61), 752.

the amount of  $\text{SiO}_2$  in the urine was low (0.007 to 0.0093 gram per day) but as he says nothing concerning the diet these figures have little significance. Similar low figures were obtained in 5 cases of cancer. He also found that while the normal human pancreas contains about 0.14 to 0.15 gram  $\text{SiO}_2$  per kilo of dry substance, in active tuberculosis the average was but 0.0828 gram, in a case of healed pulmonary tuberculosis 0.2273 gram, and in 2 chronic cases 0.2047 and 0.1835 gram per kilo. It is assumed that the pancreas plays an active rôle in silica metabolism, but no comparative analyses of other tissues are presented to support this hypothesis. However, the statement is made that the scars of healed pulmonary lesions showed no more  $\text{SiO}_2$  than normal connective tissues, but stress is laid on the reputed presence of silica in healed calcified tubercles (based on Zickgraf's analyses, discussed on p. 154).

Kahle infected guinea pigs with large doses of tubercle bacilli and gave them 0.5 to 2 grams daily of Weyland's preparation in their food. He states that after only a few days treatment there occurred a marked increase in the fibroplastic reaction in the tubercles, leading after a time to the formation of dense fibrous tissue. These changes were observed in tubercles in any and all tissues, in the liver there being a veritable cirrhosis. These anatomical observations are corroborated by Professor Rössle, who says that it was possible to distinguish the tissues of the treated animals from those of untreated controls by their anatomical characteristics without other information.

The continuance of this work was interrupted by the war, but in 1921 Kahle<sup>28</sup> made a more extended report. He agrees with Schulz that the normal human pancreas contains about 0.14 to 0.15 gram  $\text{SiO}_2$  per kilogram dry weight, and in 20 cases of tuberculosis found figures ranging from 0.01 gram in caseous pneumonia to 0.28 gram in healed tuberculosis, but in active tuberculosis the figures were usually much below normal. In pregnant women low figures were found, supposedly because of transfer of  $\text{SiO}_2$  to the fetus, perhaps accounting for the susceptibility of pregnant women to tuberculosis; in a case of puerperal miliary tuberculosis he found no  $\text{SiO}_2$  at all in the pancreas. However, in some cases of active

<sup>28</sup> Beitr. Klin. Tuberk., 1921 (47), 296.

tuberculosis he found high figures for  $\text{SiO}_2$  in the pancreas. The finding of Schulz that in tuberculosis there is no decrease in the pancreatic silica he attributes to a failure to differentiate between active and healed tuberculosis. He reports one experiment with a dog, in which the production of large wounds led to a decrease in the  $\text{SiO}_2$  excreted in the urine, attributed to retention in the new-formed connective tissue; after the pancreas was resected the urinary  $\text{SiO}_2$  was greatly increased. This experiment was not confirmed by repetition. He described 20 experiments in the silica (Weyland preparation) treatment of guinea pigs infected with large doses of human tubercle bacilli which caused a marked fibroplastic reaction about the lesions, although none of the animals was cured.

Kühn<sup>29</sup> collaborating with Kobert's laboratory in Rostok, reports that his clinical experience has indicated to him that administration of silica for long periods is beneficial in tuberculosis and also is of prophylactic value. For this purpose he has used a vegetable tea rich in silica, and also tablets containing colloidal  $\text{SiO}_2$ , which he believes is utilized, since an increased urinary excretion of  $\text{SiO}_2$  was observed after their administration, and also an increase in the blood. He believes that coincident silica and calcium treatment cannot be accomplished since insoluble compounds are produced, but he recommends alternation of the two at considerable intervals and claims to have observed favorable results, both in experimental animals and in over 400 cases of human tuberculosis.

Kesseler,<sup>30</sup> like Kühn, treated patients with a vegetable silicium (43 to 272 mgm.  $\text{SiO}_2$  per day) as well as calcium and silica tablets, and found an increase in the number of leucocytes, although not so striking an improvement in the Arneth formula as some others have described. He believes that he has seen clinical improvement "although the disease did not seem to us, as it did to Kühne, to exhibit under his therapy a course quite other than usually seen." In a case so treated that came to autopsy, however, he observed a marked connective tissue formation and encapsulation of the numerous pulmonary lesions.

<sup>29</sup> Therap. Monatsch., 1919 (33), 201; Münch. med. Woch., 1920 (67), 253; Zeit. f. Tuberk., 1920 (32), 320.

<sup>30</sup> Deut. med. Woch., 1920 (46), 239.

Helwig<sup>31</sup> says that administration of  $\text{SiO}_2$  to either men or animals causes a temporary fall in the number of leucocytes followed by a rise, especially in the polymorphonuclear forms, which show an exceptionally active phagocytic power. The Arneth formula is much improved and in Helwig's opinion there is much clinical improvement even in advanced ulcerative pulmonary tuberculosis. Unfortunately he gives no adequate details of either animal experiments or clinical observations.

Hayek<sup>32</sup> makes the following statement concerning silica therapy:

Not very pleasing are the very uncritical reports concerning the "brilliant" results from the "tea cures" that have come from the clinical side. I, myself, have never been able to observe these startling results although I have applied the silicic acid therapy very frequently in chronic consumptives.

He goes on to say that he has tried the use of subcutaneous injections of colloidal silicic acid (v. Heyden) in patients suffering for a long time with repeated small hemorrhages, and that, although the results observed so far were not convincing, they were enough to encourage further investigation.

### *Summary*

The use of silica is to be considered as in the earliest experimental stage. It seems *a priori* improbable that silica really has any particular relation to connective tissue formation and the evidence on which this theory rests is far from convincing when examined critically. Nevertheless the experimental results of Kahle are suggestive, and worthy of careful repetition. In any case, there is no reason to expect any harm whatever from this therapeutic use of silica preparations, which virtue it shares with calcium therapy.

<sup>31</sup> Med. Klin., 1921 (17), 1266.

<sup>32</sup> "Das Tuberkulose-Problem," 2nd Edition, 1921, p. 232.

## MISCELLANEOUS SUBSTANCES

*Ichthyol*

Weismayr<sup>23</sup> quotes Scarpa as declaring that ichthyol is an efficient medicine which can be placed by the side of guaiacol, equalling the latter in its influence without possessing its disadvantages. Neither has any specific influence, but both are good aids to support the hygienic and general treatment so important in tuberculosis. Many other authors have confirmed Scarpa's decision of the value of ichthyol and its derivatives—ichthyol salicylate, ichthoform, ichtharyan and others.

Ichthyol is a sulphonated distillation product of a bituminous rock which is found in the neighborhood of Seefeld in Tirol. The impressions of prehistoric fishes in these rocks make it seem probable that the oil contained in them is derived from the dead fish. Ammonium sulph-ichthyolate, briefly called ichthyol, is the form which has been used for many years in the treatment of several diseases. The sulphur has long been believed to play the chief rôle in the pharmacologic action of ichthyol and it is bound so firmly to carbon that it can not be set free without destroying the ichthyol. It is to this that ichthyol owes its reducing power and its constriction of blood vessels, thus opposing inflammations. There are no claims that ichthyol is a specific cure for tuberculosis, but that it has some bactericidal action, that it diminishes the destruction of proteins and that the patients show improved appetite, better general condition and thus increased resistance to the disease. It also facilitates expectoration, according to some writers, and stimulates respiration. According to Nolen<sup>24</sup> it is non-toxic even in large doses and non-irritant to the stomach. In spite of all that has been said for ichthyol and its compounds and derivatives, later workers seem to find but little use for them in practical clinical treatment of tuberculosis. Like so many other drugs, it has been highly praised but has been dropped almost completely. If sulphur is at the basis of whatever efficient action can be ascribed to ichthyol, it may be stated here that inhalations of pure SO<sub>2</sub> have been highly recommended for the treatment of

<sup>23</sup> Ott's Chem. Pathol. der Tuberkulose, 1903, p. 469.

<sup>24</sup> Brauer, Schröder and Blumenfeld, Handbuch der Tuberkulose, 1914.

pulmonary and laryngeal tuberculosis, but no specific efficiency has been proved and it is only claimed to facilitate expectoration.

#### *Iodin and its derivatives*

Although these have been discussed under specific chemotherapy, they may be mentioned briefly here also under general medicaments of the disease. Iodin itself, either as the tincture or in capsules, potassium iodide, iodoform and iodipin are the favorite forms in which it has been and still is used in the medical treatment of tuberculosis. Iodin in tincture form diluted with milk or other fluids is recommended in as large doses as can be borne in pulmonary tuberculosis and other forms of tuberculosis. Thus Ritter runs the dosage up gradually to 100 drops or even more three times per day. He claims that there is a marked reduction of all the sputum flora under this treatment, while the tubercle bacilli become granular and stain poorly and become reduced in number. He describes marked curative effects in bone and joint, glandular, and other forms of surgical tuberculosis, in pulmonary tuberculosis and in pleurisy with effusions, and says that it should be used in large and persistent doses to saturate tissues and inhibit bacterial growth. He also notes increased lymphocytosis as one effect of iodin treatment, thus increasing, according to Bergel's theory, the lipolytic ferment which acts on the tubercle bacilli. Escobar advises the use of tincture of iodin for direct injection into the pleura for treatment of pulmonary tuberculosis. Many writers have given favorable reports on the use of iodin and its compounds in all forms of tuberculosis. Aside from their bactericidal and fermentative action, iodin preparations are said to facilitate expectoration by increase and liquefaction of bronchial secretion.

Thus iodin preparations, whatever may be said of their specific chemotherapeutic action, are among the most persistently used and recommended of the non-specific chemical therapeutic agents. Their use on the basis of specific chemotherapy has been discussed in the preceding chapter.

#### *Saccharose*

Lo Monaco's sugar treatment of tuberculosis has been widely heralded and many reports are found in the literature of

clinical cases of tuberculosis treated by subcutaneous or intramuscular injections of saccharose. Lo Monaco<sup>35</sup> claims that subcutaneous injections of small amounts of sugar increase secretions while large amounts diminish or arrest secretions. In tuberculosis, its field is after the acute phase has passed and the main symptoms are profuse expectoration and night sweats, with little or no fever, but great debility and emaciation. In such cases, he says, the sugar treatment may induce "actual resurrection." Improvement is manifest by the tenth day. The injections do not act on the tubercle bacillus itself, but check the sputum and other excretions. When there is no further expectoration, the bacilli are supposed to die for lack of a suitable medium for growth. It is claimed that the sugar injections also modify gastric, pancreatic, biliary, renal and other secretions, as well as the bronchial. Not much animal experimentation seems to have been undertaken with the sugar treatment. However, Take-mura<sup>36</sup> injected saccharose into rabbits until diabetes developed and then inoculated them with bovine tubercle bacilli. He found that these diabetic rabbits developed a tuberculous infection much more easily than the controls. Tubercle bacilli injected into a vein or into the thoracic cavity found their way into the lungs more easily in the experimental diabetic rabbit than in the normal. The phagocytic power of the leucocytes in the serous fluid of the thoracic and abdominal cavity was found much lower in the diabetic than in the normal animal. Sugar in the culture medium neither hindered nor accelerated the growth of tubercle bacilli.

The saccharose is reported by several of the investigators to have been eliminated without being inverted and to have no effect on the life or growth of tubercle bacilli *in vitro*. It is suggested by several that the lessening of secretions and excretions, which seems to be the main effect resulting from the use of the saccharose injections, may be due to a modification of osmosis and perhaps to a vaso-motor influence.

In spite, then, of the favorable clinical reports, the small amount of experimental work does not seem to confirm them nor to put

<sup>35</sup> Presse Méd., Paris, 1918 (26), 617.

<sup>36</sup> Jap. Med. World, Tokio, 1919, No. 299.

sugar among important chemotherapeutic agents. That it has value in reducing night sweats, however, seems to have been established.<sup>37</sup>

### *Oxygen*

Since it has long been believed that fresh air, and especially air very rich in oxygen, is the best treatment for pulmonary tuberculosis, some experiments have been carried on to determine whether there is a rational basis for this belief. Rost<sup>38</sup> drained tuberculous joints by puncture, washed them out with weak iodin solution and then inflated them with oxygen. He found only one treatment necessary and the joints were then normal and functioning. In tuberculous peritonitis, the peritoneal cavity has often been filled with oxygen and psoas abscesses have been injected with oxygen, all with good results according to some clinicians. Gendron and Bouchet<sup>39</sup> have used oxygen in treatment of tuberculous fistulas and abscesses, but found no benefit unless there was an active circulation of blood through the tuberculous tissue. This was secured by superheated air, rubbing, or other measures. Then oxygen was introduced and led to rapid healing without surgical measures, under the hyperemia and oxygen.

The most extensive series of experiments has been reported by James Todd.<sup>40</sup> His experiments were carried on for five years and mostly on male guinea pigs, some 280 in all being used. The animals were inoculated with tubercle bacilli, mostly subcutaneously. They were then put in pens and oxygenized or ozonized air supplied, 1 to 4 per cent oxygen being added to the pure air. Special apparatus was devised for supplying and distributing the oxygen. His results are summarized as follows:

1. The animals oxidized before inoculation always show beneficial effects.
2. No control animal has ever survived his infection nor shown any healing.

<sup>37</sup> See Peller and Strisower, Wien. Arch. inn. Med., 1922 (3), 297.

<sup>38</sup> Indian Med. Gazette, Calcutta, 1920 (55), 329.

<sup>39</sup> Bull. Acad. Méd., 1915 (74), 819.

<sup>40</sup> Experiments with Oxygen on Disease, Monograph by James Todd, Pittsburg, 1916.

3. Treated animals have shown healing and lived longer than the controls.

4. Controls have never lived more than six months, while the treated have lived eighteen months after inoculation.

Todd believes that he has established some increased resistance to tuberculosis even in the very susceptible guinea pig.

He also ozonized olive oil and used it for feeding experiments before inoculation, apparently with some good effects.

The author admits that he does not "cure tuberculosis with the oxygen, but he believes that he can raise the vital forces of the living body by means of certain forms of intensified oxygen so as to enable that body to master its own problems."

### *Carbon dioxide*

Among the older treatments of pulmonary tuberculosis was the development of venous hyperemia and the effect of this was dependent, it was at first believed, on the bactericidal power of the blood or of the serum. Later, this was believed to be due to the carbon dioxide in the venous blood, thus corresponding with an experiment of Hamburger,<sup>41</sup> who found that blood treated with carbon dioxide had a more intense bactericidal power than ordinary blood. Based on this came a method of treating tuberculosis by developing large amounts of carbon dioxide within the body. Weber, and others, according to Weismayr, gave, a half hour before breakfast, a teaspoonful of sodium bicarbonate after a glass of water containing 12 drops of hydrochloric acid. This caused the development of much carbon dioxide which they thought exercised a healing influence on tuberculosis. Other methods were used by different authors, all with the purpose of supplying a larger quantity of carbon dioxide to the tuberculous patient.

Corper<sup>42</sup> carried on a series of experiments to test the value of this old method of treatment. He determined that 3 per cent concentration of CO<sub>2</sub> causes some inhibition of the growth of tubercle bacilli in the test tube and that 15 per cent is tuberculocidal.

<sup>41</sup> Deut. med. Woch., 1897 (23), 784.

<sup>42</sup> Amer. Review Tuberc., 1921 (5), 562.

However, tubercle bacilli will not grow in a carbon-dioxide-free atmosphere. Cultures of tubercle bacilli buried in the tissues of animals and permitted to acquire the carbon dioxide concentration of the body are definitely inhibited in their growth, while other cultures similarly buried, except that ingress of atmospheric air is permitted, show no inhibition. When viable tubercle bacilli are placed in a closed system, their growth becomes inhibited as the carbon dioxide which the organisms themselves elaborate approaches a concentration of approximately 5.5 per cent; at this concentration, respiration of the microorganisms is also reduced to a minimum. The significant feature of this study with the tubercle bacillus is that the concentration sufficient to inhibit growth in the test-tube, 5.5 per cent, occurs normally in the human body. The experiments conducted both in the test tube and in the animal body indicate that this concentration actually does inhibit the growth of the tubercle bacillus. It appears, therefore, that this factor is extremely significant in the rôle of resistance to tuberculous infection and the subsequent development of the disease in the body; that the normal body apparently possesses, by virtue of containing sufficient carbon dioxide, the ability to inhibit the growth of the tubercle bacillus.

If the conclusions from this experiment are true, we are normally immune to tuberculosis partly through the carbon dioxide content of our tissues and the disease can develop only through a reduction of its carbon dioxide content and should be readily cured by a slight increase of that content. Fact and experience however, seem to indicate that it is really not so simple as that. While Corper does not state what the carbon dioxide content of the tissues of different animals is normally, he might be able in this way to explain the different degrees of susceptibility to the disease exhibited by different species of animals and even by different animals of the same species. We await with interest further developments of this experiment.

### *Tethelin*

Tethelin, believed by Robertson to be the growth principle of the anterior lobe of the pituitary gland, has been tested by Corper<sup>43</sup>

<sup>43</sup> Jour. Infect. Dis., 1917 (21), 269.

for its chemotherapeutic efficiency in experimental tuberculosis in guinea pigs. He administered it in 25 mgm. doses on alternate days for eighteen days before infection and also during the earlier period after infection with virulent tubercle bacilli, but it had no appreciable effect on the progress of the tuberculosis nor on the duration of life of these animals. Neither did the same dose have any effect on the recession or rupture of intracutaneous tubercles produced by dead tubercle bacilli.

### *Morphine*

Corper, Gauss and Rensch<sup>44</sup> showed that 16 mgm. morphine sulphate in 10 cc. of nutrient media completely inhibited the growth of tubercle bacilli. Even saturated solutions, however, failed to kill the organisms and morphine has shown very little or no therapeutic effect.

### *Symptomatic treatment*

In addition to these we have a long list of drugs used for the various symptoms of the disease and which scarcely need mention here. Menthol has been highly recommended for tuberculous sinuses. The anaemia may be combated by iron as any other anaemia, or perhaps by arsenic. Narcotics, balsams and many complicated formulae are recommended for the cough which is so distressing a symptom of the disease. The loss of appetite and of strength are treated by the usual tonics. The fever is treated by the general hygienic, dietetic and hypurgic measures. Antipyretic drugs may, in case of need, also be used, but should be used with great care. Quinine, especially the bromide, and the salicyl preparations have been most widely recommended. While many drugs have been used to combat the night sweats which are so prominent and so unpleasant a symptom in tuberculosis, no drug therapy is now recommended for this condition, air cure, alcohol baths and such arrangements of bed and covers as prevent over-heating seeming to be sufficient.

<sup>44</sup> Amer. Review Tuberc., 1921 (5), 643.

The general and symptomatic treatment of tuberculosis, therefore, are the same as the treatment of similar conditions which are not related to a specific disease. Until a specific chemical or immunological therapy has been found for the disease, the fresh air, the hygienic and dietetic measures which have been so long and favorably known must still be relied on as the main hope in the treatment of tuberculosis.

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*Note.* The numbers printed in boldface type refer to pages upon which the topic is specifically discussed

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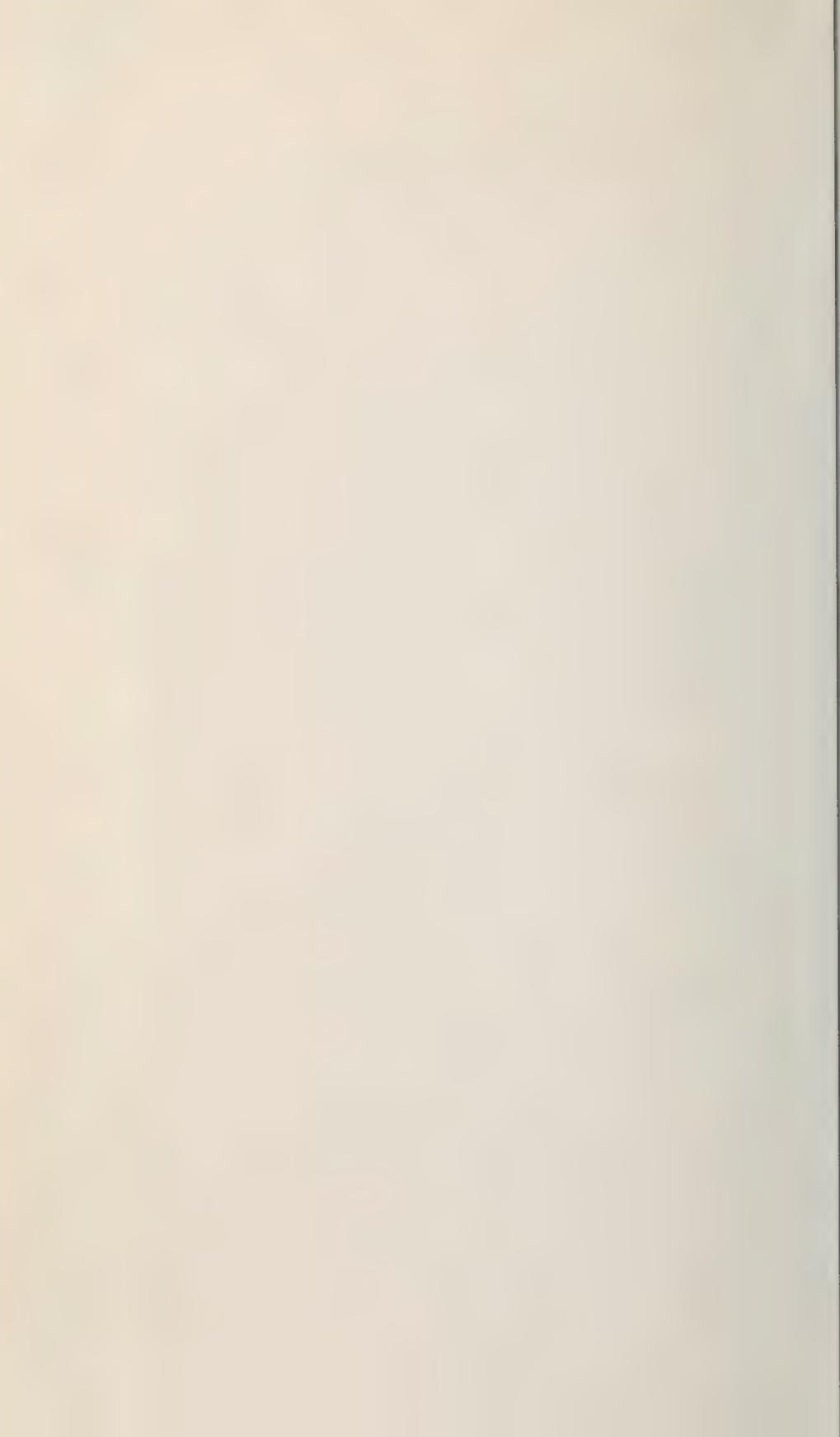
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